Tutorial Lecture

Sunday, 16th September

Main Hall

15:30 – 16:15

Tutorial Lecture 1: Nico M M Nibbering

Chair: Ryuichi Arakawa (Kansai University, Japan)

TL1-1530 Selected examples of gas-phase ion chemistry studies.

15:30 – 16:15

Nico M M Nibbering
Vrije Universiteit, Amsterdam, The Netherlands

Keywords:
tautomeric ions, time-resolved MS/MS, epimerization

Novel aspects:
Gas-phase infrared multiphoton dissociation spectroscopy and ion mobility have become in recent years new and invaluable tools for gas-phase ion chemistry studies.

Abstract:
Gas-phase ion chemistry is an area in mass spectrometry that has received much research interest since the mid fifties of the last century and there are still several groups in the world active in gas-phase ion chemistry of both positive and negative ions, either unimolecularly and/or bimolecularly.
In this tutorial lecture the formation and determination of tautomeric ion structures and intra-ionic catalyzed tautomerization in the gas phase will be discussed. In addition, an example of formation of different tautomeric structures in protic and aprotic solvents under electrospray ionization conditions will be given, as established by gas-phase infrared multiphoton dissociation spectroscopy. This will be followed by presenting an example of time-resolved MS/MS which enables to identify the structure of an ion, generated at a particular molecular ion lifetime. At the end of the lecture the power of ion mobility will be shown in elucidating the mechanism of epimerization of bis-Tröger bases having chiral nitrogen centers.

Tutorial Lecture

Sunday, 16th September

Main Hall

16:15 – 17:00

Tutorial Lecture 2: Michael L Gross

Chair: Yoko Ohashi (RIKEN, Japan)

TL2-1615

16:15 – 17:00

"Can Mass Spectrometry Play a Role in Protein Biophysics and Structural Biology?"

Michael L Gross, D Rempel, H Zhang, D Hambly, B Gau, J Chen, R Huang, J Zhang, L Jones, M Zhu
Washington University, St Louis, MO, USA

Keywords:
Protein footprinting, H/D exchange, fast photochemical oxidation of proteins

Novel aspects:
Mass spectrometry will play a role in structural biology by providing information of protein interaction interfaces, protein affinities, and protein folding, ultimately to give coarse-grained structures.

Abstract:
There is a profound need in biological sciences to determine structure and properties of proteins that do not crystallize and are not suitable for high-resolution NMR studies because the proteins are too large or they oligomerize at low concentration. Furthermore, the relevant properties of a protein are manifest in unusual media (e.g., high concentrations of lipids and other proteins) or in protein assemblies. Many low-resolution approaches exist (e.g., fluorescence, Raman, absorbance, circular dichroism, chromatography, calorimetry), but most do not readily yield information at the peptide or amino-acid levels. Mass spectrometry (MS), on the other hand, is the basis for a middle-resolution method. In appropriate experiments, MS provides a means of determining protein interactions, following folding and unfolding, and determining binding affinities and interaction sites by using chemical footprinting. Driving this approach is the wide availability of mass spectrometers, separation methods, and data processing software for analytical proteomics ; these should also be immediately applicable to protein footprinting.

To this end, we are developing fast photochemical oxidation of proteins (FPOP) and implementing HD exchange (H/DX), and other covalent modification approaches to interrogate protein interactions, interfaces, and dynamics of folding/unfolding. We are implementing H/DX to map protein interfaces and developing strategies to measure protein affinities (we call the latter approach PLIMSTEX). For irreversible labeling, we use FPOP, which has a significant advantage of speed, owing to the use of free radicals as footprinting reagents. Radical generation occurs in low nanoseconds, and the radical reactions are complete in 1 microsecond. Only a single conformation of the protein exists during the footprinting because the distribution of oxidation products is Poisson. Besides OH, other radicals can be used including the sulfate and carbonate radical anions and the iodide radical, which have different selectivities for the amino-acid residues. FPOP can also be the "probe " in a classic, two-laser "pump-probe " experiment whereby a temperature-jump perturbation is produced as a "pump " by one laser and a second laser initiates FPOP as the "probe " to afford residue-specific information. This experiment is capable of probing protein structural dynamics at the sub millisecond level with improved sensitivity and more detailed structural resolution than any physical chemical method.

In the lecture, we will describe these approaches, provide context for them in terms of other MS-based methods, and outline some applications in protein science. We will show that these approaches provide more information than fluorescence, UV absorbance, Raman, IR, nuclear magnetic resonance, and circular dichroism and may ultimately be a source for coarse-grained structures of proteins and protein assemblies.

Plenary Lecture

Sunday, 16th September

Main Hall

17:30 – 18:15

Plenary Lecture 1: Hiroyuki Hamada

Chair: Koichi Tanaka (SHIMADZU Corp , Japan)

PL1-1730 Applied Conventional Technology- Look at Tradition for Our Future-

17:30 – 18:15

Hiroyuki Hamada
Kyoto Institute of Technology, Kyoto, Japan

Keywords:
Traditional technology, Highly cultural product, Implicit knowledge

Novel aspects:
Interdisciplinary engineering which develop new ideas based on implicit knowledge of the past

Abstract:
When we think a new material, a new processing and a new product ; usually we have to develop technology, skill and soon. Fortunately, we, human, have a long history, so that there are so many traditional technologies. For example, in Japan particularly in Kyoto, there are many traditional craft works such as Urushi painting, roof tile named Kyo-gawara, and so on, because Kyoto was capital of Japan for long time, for 1200 years.
Traditional craft products give customers some kind of satisfaction when customers see the products or when they have them in their hands or use them. That is the reason why traditional craft products have been made and used for many years. We use a word “highly cultural products ” as the word that shows this kind of satisfaction. This word means the function of products and also appeals to the sensitivity of customer.
In the modern industry and production at the moment this kinds of additional value should be required. Therefore it is nice idea look at the past technologies to get some additional functions. There are appropriate words ;
Learn a lesson from the past.
However traditional technologies have been brought down as implicit knowledge. Therefore it takes long time to understand the technologies and education system is not effective. In order to overcome this problem and easily understand traditional technologies, we propose a new engineering field name as Applied Conventional Technology.
An example of applied conventional technology approach is to find secret and knack in the traditional craft works ; it is sometime material selection or sometimes fabrication method, by using scientific approach.
After this stage we can get explicit knowledge from implicit knowledge.
We can easily use the explicit knowledge to modern industry and products. Traditional craft works and processing exist in every country and every area around the world. Therefore all of scientist and fabrication Engineers are interested in this new engineering field.
The research topics undergoing are listed below ;
1) Comparison of painting technique of Urushi products between expert and non-expert
2) OBSERVATIONOF SURFACE STRUCTURE OF KYO-KAWARA (CERAMICMATERIAL FORROOF COMPONENT) FABRICATED BY TRADITIONAL SKILLFUL TECHNIQUE
3) Effects of Processing Time and Years of Experience on Pre-Bending Process for Bamboo Beam in Kyo-Chouchin (Japanese Lantern) Making Process
4) Thermal Strain of the Soldering Product in Traditional Metalwork
5) Evaluation of Pre Bending Process of Bamboo Beam in Kyo-Chochin (Japanese Lantern) Making Process
6) Motion Analysis of the “Temae ” in the Way of Tea Manufacturing movement analusis
7) Analysis of the bacterial community found in clay wall material used for the construction of Japanese traditional buildings
8) Quantification and Application of Implicit Knowledge of Bows made in Japan (Kyoto-bow)
9) Influence of proficiency in making “kanaami ”

Plenary Lecture

Monday, 17th September

Main Hall

08:00 – 08:45

Plenary Lecture 2: David E Clemmer

Chair: Peter J Derrick (Massey University, New Zealand)

PL2-0800 Development of Next Generation Ion Mobility/Mass Spectrometry Techniques

08:00 – 08:45

David E Clemmer

Indiana University Department of Chemistry/Bloomington, IN/United States

Keywords:
Ion mobility spectrometry

Novel aspects:
This is an invited lecture

Abstract:
When a pulse of ions is injected into a buffer gas and exposed to an electric field, different species will separate due to differences in their mobilities through the gas. This separation is the basis of a widely used analytical method called ion mobility spectrometry (IMS). The approach has attracted considerable attention because: 1) it allows species with identical m/z values but different shapes to be separated; 2) it can be coupled with high-speed mass spectrometry techniques to provide an additional dimension of separation without influencing analysis times; and, 3) the combined approaches provide advantages for observing low abundance ions as well as parallel fragmentation strategies. When combined with theoretical calculations the method also complements mass spectrometry as a means of determining structure. At present the resolving power of ion mobility instruments is relatively low. This talk will describe the early development of ion mobility/time-of-flight techniques and explore current efforts to improve IMS resolving power. Specifically, progress in understanding ion separation by an overtone mobility approach will be presented. This progress has driven the development of a new circular drift tube. By allowing ions to experience multiple cycles we have taken advantage of very long effective drift regions (ions have now been moved through more than 100 meters). Multipass designs facilitate a range of new experiments, including IMS^n, as well as what we call mobility ringdown experiments (where some ions are released for analysis after each cycle). The ringdown studies have been used to follow the level of H/D exchange on specific conformations of ubiquitin. The work is at a very early stage and current limitations will be presented.

Oral Session

Monday, 17th September — Main Hall

09:00 – 11:00

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation “The whole is greater than the sum of its parts” (Aristotle).

Chair: Morio Ishihara (Osaka University, Japan)

S01-0900 [Keynote Lecture] Orbitrap-based hybrid mass spectrometers: synergy of analyzers

09:00 – 09:40

Alexander A Makarov
ThermoFisher Scientific, Bremen, Germany

Keywords:
Orbitrap analyzer, high mass accuracy, ultra-high resolution, hybrid mass spectrometer

Novel aspects:
Overview of existing and future configurations of Orbitrap-based hybrid mass spectrometers and associated analytical methods

Abstract:
Orbitrap mass spectrometry has grown to become one of the most prominent techniques for high resolution, high mass accuracy analysis. To large extent this is the consequence of intricate synergy between different combinations of analyzers employed in Orbitrap-based hybrids.
Being the first high-performance mass analyzer that employs trapping of ions in electrostatic fields, Orbitrap analyzer can provide high performance analytical characteristics only when highly integrated with the ion injection process. The advent of pulsed injection from an external ion storage device has allowed the analyzer to become effectively de-coupled from previous stages of mass analysis. Therefore such a storage device in the form of the C-trap became the crucial building block of all Orbitrap-based hybrids which currently contain two major families : the original LTQ Orbitrap and the recent Q Exactive hybrids. First family employs linear traps MS^n analyzers for the first stages of mass analysis while the second one utilizes a quadrupole mass filter with hyperbolic electrodes. In both cases, preceding mass analyzer ensures optimum filling of the C-trap and thus stability of Orbitrap operation in spite of significant ion loads.
Both of hybrid families have recently entered a new phase in their short but eventful history. For the Orbitrap Elite instrument, a new construction of the analyzer with a higher field strength as well as new methods of data processing allowed to increase drastically spectral acquisition rate, improve sensitivity and performance for proteins. For the Q Exactive instrument, a variety of new methods of analysis became enabled by parallel isolation/detection as well as by multiplexing of quadrupole filtering. Performance of new instruments is described in comparison to their predecessors and high-field FT-ICR instruments. Particular attention is given to recent improvements related to protein and top-down analysis.
In conclusion, future trends and perspectives of the Orbitrap mass spectrometry are discussed. Alternative hybrid configurations are discussed from the point of view of their synergies, including ones with multiple Orbitrap analyzers operating in parallel.

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Chair: Morio Ishihara (Osaka University, Japan)

S01-0940 High Mass Resolution MALDI TOF MS/MS With A Curved Field Reflectron (Or The CFR Comes Of Age)

09:40 – 10:00

Andrew R Bowdler, Ian Brookhouse
Kratos Analytical Ltd, Manchester, UK

Keywords:
Mass resolution, MALDI TOF MS/MS, High energy CID, CFR, TOF/TOF

Novel aspects:
State of the art mass resolution for MALDI TOF MS/MS. 20keV lab energy CID with high mass resolution.

Abstract:
The curved-field reflectron (CFR) has been in existence for nearly two decades since it was introduced by Cotter and Cornish [1]. For several years the CFR was used in the only commercially available instruments capable of MALDI TOF MS/MS without scanning the reflectron. Seamless post-source decay provided advantages in terms of both sensitivity and speed of acquisition. However, the key advantage of the CFR over conventional linear field reflectrons, even those used in TOF/TOF instruments has been the ability to produce MS/MS at the full extraction energy. This is because there is no need with the CFR to re-accelerate the ions after the first TOF. So firstly, true high-energy CID is possible with lab collision energies of 20keV or more. Secondly, there is no need to discard any meta-stable ions (post-source decay) and thus very high efficiency is achieved for MS/MS.
However, even when used in MALDI TOF instruments with in excess of 20,000 mass resolution for MS, the mass resolution using the CFR for MS/MS was typically limited to isotopic resolution up to about 1000Da. This has been known for a long time to be due to the increase in the laser intensity required to produce the meta-stable ions required for MS/MS (even with high-energy CID) which is much higher than the threshold for MALDI TOF MS. Until recently, this effect had not been understood or quantified. More importantly, the reduced mass resolution has meant that the CFR instruments were not able to match the ultimate performance of other TOF/TOF instruments.
In this paper, the theory and data for a method to produce high mass resolution MS/MS with a curved field reflectron will be presented. Theoretical calculations and ion trajectory simulation data will be used to show how the reduction in mass resolution when the laser intensity is increased is due to a large increase in the initial spatial distribution in the axial direction of ions generated by the MALDI ion-source. This axial spatial distribution is passed on by the parent ions during meta-stable decay or CID to the fragment ions. It will be shown that it is possible to gain MS levels of mass resolution for MS/MS if this axial spatial distribution is re-focussed before all of the fragment ions reach the entrance to the curved-field reflectron. With this technique and in combination with the curved-field reflectron, fragment mass resolutions in excess of 10,000 can be achieved for MS/MS of meta-stable ions and CID at lab energy of 20keV. By using axial spatial distribution focusing (ASDF), the curved-field reflectron is again capable of defining the state of the art for MALDI TOF-MS/MS and can really be said to have come of age.

[1] Cornish TJ and Cotter RJ 'Non-linear field reflectron' US patent 5464985

Oral Session

Monday, 17th September

Main Hall

09:00 – 11:00

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

Chair: Morio Ishihara (Osaka University, Japan)

S01-1000

10:00 – 10:20

High resolution, high aperture, high sensitivity TOF mass-spectrometers with expanded functionalities

Aldan A Sapargaliyev[3], Igor F Spivak-Lavrov[2], Yerbol A Sapargaliyev[1,3]

[1]Scientific Center "REB", [2]Aktobe State University, Aktobe, Kazakhstan, [3]Scientific Center REB, Almaty, Kazakhstan

Keywords:
TOF, instruments, parallel tandem, high sensitivity, high resolution

Novel aspects:
Novel designs of compact TOF analyzers with the longest flight-paths (TOF) are suggested. Ion-beam channels utilize parallel tandem analysis simultaneously by energy and mass in the "nested time " mode.

Abstract:
The set of novel ion-optical (IO) designs of Time-of-Flight (TOF) analyzers with a loop ion trajectory formed by the Wedge-Type-Two-Dimensional (WT) grid less mirrors is proposed [1]. These TOF analyzers possess record characteristics twice as much overcoming their already known analogues by resolution and sensitivity. They can be used in mass-spectrometers of different configurations (tandem, hybrid) for the preliminary selection of ions and/or for the final division of the flow by masses. Furthermore, they feature extended functional capabilities enabling multi-object (simultaneous separate analysis of ion beams from two and more different sources) and multi-parameter mass-spectrometry. Exceptional features of the TOF analyzers proposed, including the high sensitivity, are due to the following innovations.
WT mirrors are made in the form of longish sheets which create two-dimensional electric field with the middle plane. Providing the ion beam loop configuration, the disposition of mirrors in the analyzer allows reimbursing the transverse spatial dispersion by energy, which appears at every reflection of the ion beam. The plane of the loop trajectories of the ion beam moves along the longish sheets providing the increase of the unlooped path of ions. The use of various three-dimensional IO reflection elements allows spatially to focus the ion beam in two transverse directions and thus to transport ion packages without significant loss. Compared to axis symmetric IO elements, the middle plane IO elements have smaller aberrations and larger aperture, which enables mass-spectrometers with high resolution and sensitivity. Meanwhile, the record-breaking characteristics can be achieved while using the multilayer-multi-reflective TOF analyzers. The use of mass-spectrometers with TOF analyzers for parallel mass spectrometry of two or more tract ion beams is stipulated, that provides the expansion of their functionality and also increases the speed of receiving mass-spectrometry data and enhances the credibility of the results.
Some of the offered designs, including multi-channel, hybrid and tandem, provide possibility of simultaneous analysis of the ion beam by energy and mass.
The proposed designs suggest the longest fly-distance (TOF) at the smallest sizes of ion channel. Resolution of mass-spectrometers may vary from tens of thousands (two-reflective) to several millions (multilayer-multi-reflective). For example, single-layer-multi-reflective TOF system at dimensions 0.4x0.2x0.05m 3 (layer width 0.05m) provides 32m path-of-flight (resolution 200,000). Five-layer-multi-reflective system (dimensions 0.4x0.2x0.25m 3) provides 160m path-of-flight (resolution 1,000,000). In general, the TOF analyzer can contain any number (ten and more) of layers.
Suggested variants of ion-beam channels enable utilizing the parallel tandem TOF analysis in the "nested time " mode, in the better way than the ones suggested by other systems.
The general method of investigation and the corpuscular-optical theory of the spatial and temporal structure of charged particle flows in systems including in elements with special points (mirrors and emission units) can be found in works [2-4]. The methods and theory developed in these works are the basis for the theoretical substantiation of TOF analyzers proposed.

[1] Sapargaliev A.A., The method of mass-spectrometry (variants) and the device for its realization, International publication No. PCT/KZ2011/000011, Pub. No.: WO/2012/005561, International Filing Date: 08.07.2011, Publication Date: 12.01.2012.
[2] Sapargaliev A.A., Yakushev E.M., Osnovyobshego metoda teorii fokussirovki zariazhennykh chastits, (Fundamentals of the general method of the theory of focusing of charged particles) Izv.AN KazSSR, seria Phyz.mat. 1980, 2, pp. 22-26.
[3] Yakushev E.M., Sapargaliev A.A., Elengeev A.K., Obshaya Teoria prostranstvennoy I vremiaproletnoi fokussirovki zariazhennykhchastits v statsionarnykh electromagnitnykh poliakh (General theory of spatial and time-flight focusing of charged particles in stationary electromagnetic fields). ZhTF, 1985, V.55, 7, p.1291-1299.
[4] Bimurzaev S.B., Yakushev E.M., Electromagnetic mirror objective with removable spherical aberration, Nucl. Instr. Meth. in Phys. Res., 2011, V. A 645, p. 33-34.

Oral Session

Monday, 17th September — Main Hall

09:00 – 11:00

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

Chair: Morio Ishihara (Osaka University, Japan)

S01-1020
10:20 – 10:40

On the way to the holy grail of CID (20 keV and C12 precursor ion): from MALDI-LinTOF/CF-RTOF- to MALDI-SpiralTOF/offset parabolic-RTOF-MS

Guenter Allmaier[1], Ernst Pittenauer[1], Pavel Rehulka[2], Akihiko Kusai[3]

[1]Vienna University of Technology, Vienna, Austria, [2]University of Defence, Hradec Kralova, Czech Republic, [3]JEOL (Europe), Croissy-sur-Seine, France

Keywords:
MALDI, High-energy CID, Spiral TOF/Parabolic RTOF, Triglycerides, Glycerophospholipids

Novel aspects:
A MALDI tandem MS (spiralTOF, HE (20 keV) -CID, parabolicRTOF) can be seen as reaching the holy grail of CID structural analysis allowing C12 ion selection/true HE-CID of lipids out of mixtures.

Abstract:
With the introduction of MALDI-TOF/RTOF-technology during the turn of the 21st century high-energy (beyond or at 1 keV) collision-induced dissociation (HE-CID) of biomolecules (e.g. lipids, peptides, carbohydrates) for structural elucidation was back in business after the slow disappearance of classical high performance tandem four sector instruments due to sensitivity issues and the complexity of the mass spectrometer compared to modern tandem or multi stage mass spectrometric instrumentation (IT, QqRTOF and QqQ) . Whereas tandem four sector instruments (for example EBEB (JEOL HX110/HX110) configurations) offered monoisotopic (C_{12}) precursor ion selection and keV-collision regimes (rich in product ion formation across the whole m/z range)) at reasonable resolution the latter tandem mass spectrometric instrumental developments (IT, QqRTOF and QqQ) allowed only eV-collisions frequently poor in formation of structure diagnostic product ion formation. Major draw-backs of recent HE-CID TOF/RTOF-instrumentation particular for lipid analysis are mainly based on technical limitations in MS 1 and the associated ion gate-technology for precursor ion-selection thus never enabling clear monoisotopic precursor ion selection. This is extremely important as the smallest mass difference between two lipid species is often just 2 Da (i.e. one double bond) . Therefore monoisotopic precursor ion selection (mass window 1 Da without any isotope interferences at suitable sensitivity) is urgently needed on routine base for unambiguous lipid species characterization and identification under "true " HE-CID (E_{LAB} = 20 keV) conditions.
As recent advancement (i.e. coming close to the holy grail of CID structural analysis) the introduction of a MALDI tandem HE (20 keV) -CID MS consisting of a spiralTOF as MS 1 and an offset parabolic RTOF as MS 2 can be seen allowing again C_{12} (monoisotopic) precursor ion selection at good sensitivity. For evaluation of this latter, most advanced device samples of selected triacylglycerols (TAGs from castor bean and linseed oil) and glycerophospholipids (GPL) from various classes (PC, PE, PS, PI, etc.) were analyzed by means of the JMS-S300 SpiralTOF (JEOL) and including various other TOF/RTOF-instruments from different manufacturers (ABI 4700 and 4800 proteome analyzer (both : ABSciex) and Axima TOF^2 (Shimadzu Kratos Analytical) . As precursor ions $[M+H]^+$ and $[M+nNa\ (n\ 1)\ H]^+$ ions (n = 1 - 3) were selected for positive ion and $[MH]^-$ for negative ion mode. All product ion assignments were based on previously published product ion nomenclature for TAGs and GPLs. It turned out that the new device allowed clear monoisotopic precursor ion selection out of complex lipid mixtures at good sensitivity and 20 keV HE CID fragmentation of these ions for detailed structure elucidation (i.e. double bond location) of lipids across the complete m/z range of interest.

Oral Session

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09:00 – 11:00

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

Chair: Morio Ishihara (Osaka University, Japan)

S01-1040
10:40 – 11:00

Identification of trace level process related impurities of small molecule Irbesartan, an angiotensin II receptor antagonist through MSMS analysis

Saravanan Subramaniam, Raman Palvannanathan, Rampriya Uthayakumar, Govindarajan Chandramohan, Mohan Kasi, Arvind Thyagarajan, Manohar Venkat

Indian Institute of Chromatography & Mass Spectrometry

Keywords:
Irbesartan, trace level impurities, SSSfunction

Novel aspects:
The novel features of the MS technology namely Synchronized Survey Scan (SSS) has been exploited to characterize process related unknown impurities at a concentration of less than 0.10%.

Abstract:
Identification of impurities based on process parameters is one of the regulatory requirements to ensure the safety of the final drug substance. If the impurities are not process related, but new ones, it is imperative that one has to establish the biological toxicity of the unknown impurities, which in turn will be highly cost prohibitive. The MSMS technology available in triple quadrupole mass spectrometer with multiple collision energy features becomes very much useful to identify these process related / unknown impurities, which are present in trace level in drug substances. This paper presents the identification of five unknown impurities of Irbesartan an angiotensin II receptor antagonist molecule. The MSMS analysis demonstrates that these five impurities are process related only and not new unknown impurities.

Irbesartan was in-house synthesized at Indian Institute of chromatography and Mass Spectrometry (IICMS) , Chennai, India. The purity of the compound was found to be 99.21% (Area normalization method) . The concentration of five impurities was found to be varying from 0.04% to 0.32% (Area normalization method) .

The salient features of LCMS 8030 like Synchronized Survey Scan (SSS) provides the MS and MSMS data simultaneously in a single run and paves a way for rapid identification of impurities. Further the simultaneous MS analysis in both positive and negative modes, MSMS analysis with different collision energies and MSMS analysis with neutral loss function have enabled us to characterize the impurities of Irbesartan.

The molecular ion of the acive compound Irbesartan has been observed as m/z 429 in ESI positive mode, confirmed in the simultaneous ESI negative mode analysis as m/z427. The MSMS analysis of the main compound irbesartan in ESI positive ionization shows the charecteristic fragmentation ions such as m/z 429, m/z 207, m/z 195, m/z 180 and m/z 84.

It is intresting to note the MSMS features of LCMS 8030 shows the charecteristic neutral loss resulting to the presence of ion m/z 207. Simillar charecteristic ions of the impurities were also observed in the MSMS mode. The MSMS analysis have been carried out at the collision energies same as that of the main compound and the data analysis has enabled us to observe the presence/ absence of methyl group leading to the formation ofimpurity- 2 and impurity-5. All the impurities except impurity- 3 have their origin from the raw meterial spiroketones used in the synthesis of irbesartan. Impurity- 3 is already reported as USP impurity-A.

The entire analyses show how the MSMS architecture available in LCMS 8030 can effectively be employed to characterise the process related impurities. The unique feature of this capability is the identification of the molecular ion at very low concentration.

Oral Session

Monday, 17th September

Room A

09:00 – 11:00

Session 2: Advances in Methods and MS Instrumentation for Biomolecule Characterization

Chair: Vicki H Wysocki (Ohio State University, USA)

S02-0900

09:00 – 09:40

[Keynote Lecture] Chemical Cross-Linking and Mass Spectrometry: A Fruitful Combination for Protein 3D-Structure Analysis

Andrea Sinz

Martin-Luther University Halle-Wittenberg, Halle, Germany

Keywords:
Chemical cross-linking, ESI-LTQ-Orbitrap-MS/MS, protein 3D-structure

Novel aspects:
Combination of chemical cross-linking and MS for protein structure analysis.

Abstract:
Detailed knowledge of three-dimensional protein structures and the identifcation of protein-binding partners are critical for understanding cellular processes at the molecular level. However, applying conventional methods of structural biology is challenging when analyzing membrane proteins, transient complexes or very large protein assemblies. Chemical cross-linking in combination with mass spectrometry (MS) and computational modelling has emerged as an alternative strategy to obtain three-dimensional structural information of proteins and protein complexes [1-3]. The chemical cross-linking/MS approach can be used in combination with other low-resolution structural methods to study proteins and protein assemblies, which are otherwise not amenable to the high-resolution structural techniques of X-ray crystallography or NMR spectroscopy. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-linking data, three-dimensional structural models of proteins and protein complexes can be constructed. We employ the chemical cross-linking/MS strategy to study proteins and protein complexes with a wide range of biological activities. The protein systems currently under investigation in our lab comprise the peroxisome proliferator-activated receptor alpha, the formate channel FocA, as well as calmodulin/Munc13 and laminin/nidogen complexes. Most commonly, homo-bifunctional amine-reactive cross-linkers, such as *N*-hydroxysuccinimide esters, are used for studying protein assemblies. Our aim is to extend the arsenal of exisiting cross-linkers to obtain complementary structural information. We explore the incorporation of unnatural photo-reactive amino acids into the proteins of interest, and we use specifically designed cross-linkers, which are cleaved by collisionally activated dissociation (CAD) conditions during MS analysis [4]. After the cross-linking reaction, the chemically modified proteins are enzymatically digested and the resulting highly complex peptide mixtures are analyzed by MS (nano-HPLC/MALDI-TOF/TOF-MS/MS and nano-HPLC/nano-ESI-LTQ-Orbitrap-MS/MS). Mass spectra are screened for cross-linked products by the StavroX software [5] and the distance restraints we obtain serve as the basis for constructing structural models of the proteins under investigation using Rosetta. With the continual improvements in mass spectrometric equipment and bioinformatics tools, the chemical cross-linking strategy can be expected to greatly facilitate the 3D-structural analysis of proteins.

Literature References :
[1] Sinz, A., *Mass Spectrom. Rev*. **2006**, *25*, 663. [2] Leitner, A. *et al.*, *Mol. Cell. Proteomics*. **2010**, *9*, 1634. [3] Rappsilber, J., *J. Struct. Biol*. **2011**,*173*, 663. [4] Muller, M.Q. *et al.*, *Anal. Chem*. **2010**, *82*, 6958. [5] Gotze, M. *et al.*, *J. Am. Soc. Mass Spectrom.***2012**, 23, 76.

Oral Session

Monday, 17th September

Room A

09:00 – 11:00

Session 2: Advances in Methods and MS Instrumentation for Biomolecule Characterization

Chair: Vicki H Wysocki (Ohio State University, USA)

S02-0940

09:40 – 10:00

Applications of online- bioaffinity- mass spectrometry to structure and affinity determination of neurodegenerative proteins from brain material

Stefan Slamnoiu[1], Camelia Vlad[1,2], Adrian Moise[1], Mihaela Stumbaum[1,2], Thomas Gronewold[2], Markus Perpeet[2], Michael Przybylski[1]

[1]Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, Department of Chemistry, University of Konstanz, Germany [2]SAW Instruments GmbH, Bonn, Germany

Keywords:
affinty mass spectrometry, SAW-ESI-MS, biosensor, neurodegenerative proteins, alpha synuclein

Novel aspects:
The new affinity-MS method based on the online coupling of SAW-biosensor to ESI-MS provides information of biopolymer interactions at molecular level, enabling affinity detection and quantification, and ligand structure identification.

Abstract:
Introduction:
In the analysis of affinity interactions biosensors are a key analytical tool, being able to detect and quantify bioaffinity bindings. Nonetheless, biosensors do not provide the chemical structure identification of ligands, for which mass spectrometry is a powerful tool [1]. Here we show that the online combination biosensor- mass spectrometry provides simultaneous chemical structure identification and quantification of bioaffinity interactions.

Methods:
In this study we report affinity interaction and structural studies of antigen-antibody and peptide-protein complexes, as well as of biological samples: mouse brain homogenate containing α-Synuclein and human CSF containing Aß peptides. The SAW-biosensor was directly coupled to (i) ESI ion trap, and (ii) ESI FT-ICR-MS. The analysed complexes included Aß (1-16) peptide / anti- Aß (1-16) antibody; Aß(12-40) / anti- Aß (17-28) antibody; Tau protein (isoform 2N/4R) / anti-Tauspecific antibody (clone TAU5); melitin / calmodulin, and α-Synuclein / anti- α-Synuclein antibodies. An anti- α-Synuclein antibody was used for the affinity characterisation of mouse brain homogenate, while anti- Aß antibodies were employed in the analysis of human CSF. Antibodies / proteins were immobilised via a thiol linker on the surface of a gold chip inside the biosensor, and their interactions with the specific peptide or protein were monitored with the SAW biosensor. Using the online interface developed in our laboratory, the bound peptide or protein was eluted at acidic conditions from the gold chip and analysed by ESI mass spectrometry, which provided the structural characterisation of the ligands [1].

Results:
The online combination of SAW biosensor and mass spectrometry allowed the structural identification and affinity determination of affinity-bound Tau, Aß and α-Synuclein. The mass spectrometric characterisation provided details on post translational structure modifications of proteins and peptides present in biological samples, while the analysis of the binding kinetics delivered the KD values for the antibody-antigen interactions. The key achievement of the method consists in the ability to structurally characterize specific biopolymer molecules (Aß, α-Synuclein), occurring at low concentrations in biological material. Using this new hybrid tool, screening for biomarkers of neurodegenerative diseases will become more efficient and accurate, leading to easily quantifiable and interpretable results.
(Supported by grant KF2026662 from the Bundesministerium für Wirtschaft und Technologie, Berlin Germany)

[1] Dragusanu, M. et al. *J. Am. Soc.Mass Spectrom*. 21, 2010, 1643-1648.

Oral Session

Monday, 17th September

Room A

09:00 – 11:00

Session 2: Advances in Methods and MS Instrumentation for Biomolecule Characterization

Chair: Vicki H Wysocki (Ohio State University, USA)

S02-1000

10:00 – 10:20

Pinpointing individual sites that change conformation in large protein complexes by H/D exchange and ETD-MS

Song Hongjian[1], Jeff M Brown[2], Steven D Pringle[2], Michael Morris[2], Kasper D Rand[1]

[1]Laboratory for Biomacromolecular Analysis, Advanced Drug Analysis Section, Department of Pharmacy, University of Copenhagen, Denmark, [2]Waters MS Technologies Centre, Manchester, UK.

Keywords:
Hydrogen/deuterium exchange ; protein conformation, conformational changes, protein-protein complexes, electron transfer dissociation

Novel aspects:
The ability of mass spectrometry to obtain single-residue structural information even for large and challenging protein complexes of therapeutic interest.

Abstract:

Introduction

Hydrogen/deuterium exchange (HDX) analysis of individual residues in proteins by electron transfer dissociation (ETD) has paved the way for MS analysis of protein structure at an unprecedented level of detail [1,2] . HDX-ETD experiments on large protein systems [3] however remain a challenge as these yield highly complex peptide mixtures for which optimal ETD spectra can be difficult to acquire on a chromatographic timescale. Here we combine optimized ETD with chromatographic separation in an online HDX-ETD workflow to successfully obtain site-specific HDX information on the therapeutically important 75KDa protein complex formed by Coagulation Factor VIIa (FVIIa) and Tissue Factor (TF) during blood coagulation [4] . Our results demonstrate the ability of mass spectrometry to obtain single-residue structural information even for large and challenging protein complexes of biopharmaceutical interest.

Methods

Deuterium labeled samples of recombinant FVIIa and TF were prepared by an automated liquid handling robot (LEAP technologies) . Labeled protein samples were loaded onto a refrigerated nanoACQUITY UPLC system (Waters Inc.) interfaced with a Synapt G 2 mass spectrometer (Waters Inc.) for pepsin digestion, chromatography and mass spectrometry. The mass spectrometer was equipped with an ESI source operated at predefined settings for minimal H/D scrambling as described previously [5,6] . ETD was performed in the trap T-wave by reacting radical 1,4 dicyanobenzene anions with quadrupole-selected deuterium labeled peptide ions.

Results

Pepsin proteolysis of FVIIa-TF samples yielded a complex mixture of overlapping peptides and 57 unique peptides covering 95% of the sequence of FVIIa were identified. HDX-MS experiments performed without ETD on free and TF-bound FVIIa revealed a subset of 11 representative FVIIa peptides that displayed reduced deuterium uptake upon TF binding and thus contained residues whose conformation was affected upon TF-induced activation of FVIIa. HDX-ETD experiments on the hybrid Q-TOF platform were performed by use of a data-dependent method to optimize precursor selection and ETD acquisition parameters for the 11 FVIIa peptides of interest based on their respective elution time and most abundant charge states observed. Further, mild supplemental activation of ETD product ions performed in a data-dependent manner in the transfer T-wave was found to improve ETD efficiency for 2+ peptide ions. Using the tailored HX-ETD workflow, we have extracted site-specific HDX information for FVIIa when bound to TF which has allowed us to pinpoint, for the first time, a network of residues in FVIIa that relay a concerted structural change from the TF binding site of FVIIa to the active site region of the protease. This provides detailed molecular insight into the allosteric regulation of this enzyme and key information for the design of variants of FVIIa with improved activity and biopharmaceutical efficacy. Our results demonstrate that ETD can be performed online on a chromatographic timescale using a hybrid Q-TOF platform in an improved HX-ETD type workflow that is poised to open up for high-resolution HDX analysis of

large and challenging protein systems of key biological and pharmaceutical interest.

References

[1] Rand, K. D., Zehl, M., Jensen, O. N. & Jorgensen, T. J. D. *Anal. Chem.* 2009, 81, 5577-5584

[2] Zehl, M., Rand, K. D., Jensen, O. N. & Jorgensen, T. J. D. *J. Am. Chem. Soc.* 2008, 130, 17453-17459

[3] Huang, R. Y. C., Garai, K., Frieden, C. & Gross, M. L. *Biochemistry* 2011, 50, 9273-9282

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[6] Rand, K. D., Pringle, S. D., Morris, M., Engen, J. R. & Brown, J. M. *J. Am. Soc. Mass Spectrom.* 2011, 22, 1784-1793

Oral Session

Monday, 17th September — Room A

09:00 – 11:00

Session 2: Advances in Methods and MS Instrumentation for Biomolecule Characterization

Chair: Vicki H Wysocki (Ohio State University, USA)

S02-1020 What happens on the histone multimers in the gas phase?

10:20 – 10:40

Kazumi Saikusa[1], Sotaro Fuchigami[1], Kyohei Takahashi[1], Yuuki Asano[1], Aritaka Nagadoi[1], Hiroaki Tachiwana[2], Hitoshi Kurumizaka[2], Mitsunori Ikeguchi[1], Yoshifumi Nishimura[1], Satoko Akashi[1]

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Keywords:
intrinsically disordered protein, histone H2A/H2B dimer, histone H3/H4 tetramer, electrospray ionization ion mobility mass spectrometry, molecular dynamics simulation.

Novel aspects:
First application of IM-MS in combination with MD simulation to characterize the structural varieties of histone multimers with disordered tail regions

Abstract:
Gas-phase behaviors of various protein complex ions have been characterized by electrospray ionization mass spectrometry (ESI-MS) in combination with ion mobility (IM) separation. In the present study, we have investigated gas-phase structures of histone multimers, which have a folded core structure with disordered tail regions, by ESI-IM-MS and molecular dynamics (MD) simulation. Histone proteins are chief components of nucleosome core particle (NCP), the basic structural unit of eukaryotic chromatins. NCP consists of approximately 147 base pairs of DNA wrapping around a histone octamer, $(H2A/H2B/H3/H4)_2$, and its assembly and disassembly is a dynamic process that regulates DNA transcription, replication, and repair. In order to understand the mechanism of the NCP assembly and disassembly, structural characterization of the histone multimers, H2A/H2B dimer and H3/H4 tetramer, should be of great help. However, their atomic-level structures have not been determined because of the high flexibility of the N-terminal disordered tail region of each histone protein. Thus we have characterized these histone multimers by MS and MD simulation.
Human histone H2A/H2B dimer and H3/H4 tetramer were prepared by refolding of the recombinant proteins. When the sample solutions were prepared in an extremely high concentration of ammonium acetate and subjected to ESI-MS, the intact histone multimer ions were successfully observed without dissociation into each monomer. In the IM-MS analysis, experimentally obtained arrival times of these histone multimer ions presented rather wide distributions, suggesting that they were in various conformations.
In order to characterize the structures of these histone multimers observed by IM-MS, consecutive MD simulation for these histone multimers was carried out first in aqueous environment then in vacuo. MD simulation in aqueous solutions containing high salt concentrations for 10 ns revealed the structures of histone multimers with stable folded core parts and disordered tail regions. The 10 simulated structures every 1 ns in solution were then subjected to the gas-phase simulations. The behaviors of the histone multimers in vacuo were analyzed by MD simulation with attaching 11+ (H2A/H2B dimer) or 16+ (H3/H4 tetramer) charges, which were the dominant charge states observed in the ESI mass spectra, to the initial solution structures. When the distribution of the calculated CCS values for 150 simulated structures of each histone multimer was analyzed, it was found that the MD simulated structures at the ESI-charge state exhibited a relatively narrow CCS distribution, which was not completely consistent with the results obtained by IM-MS. Therefore, another set of MD simulation of the histone multimers at the Native-charge state, i.e., the charge state that was employed in the MD simulation in aqueous environment, was also carried out. It was found that the MD simulated structures at the Native-charge state demonstrated a wide CCS distribution, which was similar to that observed in the IM-MS experiments. In addition, detailed analysis of the simulated structures revealed that the wide CCS distribution was derived from structural variety of the tail regions in the gas phase.
This study indicates that the combination of IM-MS and MD simulation enables comprehensive characterization of the gas-phase structures of biomolecular complexes containing disordered regions. Further discussion on the transition of the structures during ES ionization process will be carried out based on the results of IM-MS and MD simulation.

Oral Session

Monday, 17th September — Room A

09:00 – 11:00

Session 2: Advances in Methods and MS Instrumentation for Biomolecule Characterization

Chair: Vicki H Wysocki (Ohio State University, USA)

S02-1040
10:40 – 11:00

Dibasic Site-Specific Proteolysis for Improved Bottom-Up and Middle-Down Proteomics

Unige A Laskay[1], Upir Oxana[1], Luca Fornelli[1], Kozhinov N Anton[1], Michel Monod[2], Goran Mitulovic[3], Yury O Tsybin[1]

[1]Ecole Polytechnique Federale de Lausanne Switzerland, [2]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [3]Medical University Vienna, Austria

Keywords:
Middle-down proteomics, proteomics profiling, supercharging, high resolution

Novel aspects:
We present a summary of our efforts focusing on different steps of the proteome analysis pipeline, starting from protease characterization, sample preparation, LC separation, ionization to data acquisition and analysis.

Abstract:
In-depth bottom-up mass spectrometry (MS) -based proteomics profiling is currently limited by the effective dynamic range of the proteins present and the under-sampling of the peptides generated by proteolysis. Nonetheless, it allows for identification of several hundred, or even thousands of proteins in a chromatographic run, while top-down proteomics is successful in detecting and analyzing multiple isoforms and modification sites on the same protein. It can be envisioned, that by decreasing the frequency of cleavage sites of a protease, the average size of the generated peptides will increase, and consequently, the sample complexity decreases. Middle-down proteomics (MDP) is a recently emerged approach that targets exactly this hypothesis by combining the benefits of bottom-up and top-down techniques and it is fully compatible with the scan speed of the state-of-the-art FTMS instrumentation operating in LC-MS/MS mode. Here, we advance bottom-up and middle-down proteomics by judicious selection of endoproteases capable of providing increased sequence coverage and LC-MS/MS method tailoring to the novel proteolytic peptide properties to deliver the required improvement in protein identification and characterization.
Trypsin and LysC are the two most commonly used proteases in bottom up proteomics ; the benefits of having a basic residue on the C-terminus for the quality of the MS/MS data have been extensively studied. Much less studied, dibasic site-specific proteases cleave at adjacent K and/or R sites, where cleavage may occur before, after or between the two basic residues, therefore resulting in longer peptides carrying multiple charges at one or both termini. To evaluate the applicability of dibasic proteases for MDP, peptides mimicking these theoretical protease products were synthesized by Fmoc solid state peptide synthesis and their HCD and ETD fragmentation behavior was compared. We have determined optimal fragmentation conditions using direct infusion of these synthetic peptides by comparing the sequence coverage obtained at different HCD collision energies or ETD reaction times for the different charge states. All MS/MS experiments were performed on a high-field compact Orbitrap Elite mass spectrometer (Thermo Scientific) , with resolution setting of 60k (at *m/z* 400) for precursor ion scanning and 15k for product ions using the eFT function enabled.
Another practical requirement for dibasic proteases is the availability, ease and cost of large scale production. We have found two proteases, Sap 9 and Sap10 that are specific to dibasic sites and cleave after two adjacent basic residues ; these were expressed in *Pichia pastoris* and His-tag purified ; a green fluorescent enzyme assay activity kit was used to determine the optimal working conditions. We have found that the enzymes are most active at acidic pH conditions, at short incubation times.
Nano-LC separation was performed on a Dionex UPLC 3000 system (Thermo Scientific) on a 25 cm C18 column. Solvent and gradient optimization was performed on the synthetic peptides. We have found that the presence of methanol in the mobile phase causes an even distribution of the peptides over the LC gradient resulting in peak broadening ; this is expected to allow for more sampling time for co-eluting peptides and increased MS/MS efficiency. To increase the intensity of the highly charged precursor ions we have used an in-house built dual-spray microchip obtained through collaboration. Importantly, ion supercharging occurs in-spray ; [1] therefore it has no detrimental effect on the chromatographic separation.
Our preliminary data indicates that commercial bioinformatics software designed for bottom-up experiments cannot be readily implemented due to the maximum *m/z* constraint for the peptide precursor ion and because the advantage of high resolution MS/MS data is not fully exploited. We thus implement software tailored for top-down data analysis for interpretation of the MDP data.

[1] Miladinovic S.M., et al., *Analytical Chemistry* (2012) , in print

Oral Session

Monday, 17th September

Room B-1

09:00 – 11:00

Session 3: Structures and Dynamics of Atomic and Molecular Clusters

Chair: Fuminori Misaizu (Tohoku University, Japan)

S03-0900

09:00 – 09:40

[Keynote Lecture] Recent Advances in the Vibrational Spectroscopy of Mass-Selected Gas Phase Cluster Ions

Knut R Asmis

Fritz-Haber-Institut der MPG, Berlin, Germany

Keywords:
infrared photodissociation, clusters, ion trap, messenger-tagging, isomer-selective

Novel aspects:
(1) Measurement of infrared photodissociation spectra of thermalized mass-selected cluster ions. (2) Isomer-selective photodissociation spectra using population-labeling IR/IR double resonance spectroscopy combined with a triple mass spectrometer.

Abstract:
There is an ongoing effort, spanning many branches of the physical sciences, to characterize the structure of gas phase ions, ranging from simple polyatomic species all the way to large biomolecules containing many thousand atoms. Several techniques have been refined over the past decade, of which infrared photodissociation (IRPD) spectroscopy has evolved to be one of the most powerful and widely applicable ones. I will give an overview of IRPD experiments performed in our group, focusing on the structural characterization of mass-selected cluster ions, and how these results can help in our fundamental understanding of heterogeneous catalysis as well as ion solvation.

Due to their structural variability and redox activity transition metal oxides are widely used in heterogeneous catalysis. However, in most supported catalysts, neither the size and distribution of the active particles on the support surface, nor their structure is sufficiently known. Therefore it proves helpful to study the size-dependent properties of such particles in the gas phase, i.e., in the absence of any interactions with a medium or support. Our ongoing IRPD experiments on transition metal oxide clusters are aimed at gaining a molecular level understanding of their structure-reactivity relationship and ultimately unravelling the adsorbate/active site/support interaction important in heterogeneous catalysis.

Understanding how protons are hydrated is another important and challenging research area. The anomalously high proton mobility of water can be explained by a periodic isomerization between the Eigen and Zundel binding motifs, $H_3O^+(aq)$ and $H_5O_2^+(aq)$, respectively, even though the detailed mechanism is considerably more complex and still not fully understood. These rapidly interconverting structures from the condensed phase can be stabilized, isolated and studied in the gas phase in the form of protonated water clusters. The smallest protonated water cluster that exhibits structural isomers related to both motifs (Eigen and Zundel) experimentally is the protonated water hexamer $H^+(H_2O)_6$. Here, results on isomer-selective double resonance experiments on $H^+(H_2O)_6$ are presented. Protonated water clusters are formed by electrospray ionization, mass-selected, cooled to cryogenic temperatures, and messenger-tagged with H_2 in a buffer gas filled ion trap. Isomer-selective photodissociation spectra are measured from 300-4000 cm^{-1} using population-labeling IR/IR double resonance spectroscopy combined with a triple mass spectrometer.

Oral Session

Monday, 17th September

Room B-1

09:00 – 11:00

Session 3: Structures and Dynamics of Atomic and Molecular Clusters

Chair: Fuminori Misaizu (Tohoku University, Japan)

S03-0940

09:40 – 10:00

Laser desorption supersonic jet spectroscopy of neurotransmitter molecules and partial peptides of their receptors

Shun-ichi Ishiuchi, Masaaki Fujii

Tokyo Institute of Technology, Yokohama, Japan

Keywords:
laser desorption, supersonic jet, IR spectroscopy, neurotransmitter, peptide

Novel aspects:
Specifically small numer of conformers of catecholamines in gas phase is found by uniquely improved laser desorption supersonic jet spectroscopy.

Abstract:
The neural signal is transmitted by specific molecules known as neurotransmitters, which bind to receptor protein, from one neuronal cell to the next, through the accurate molecular recognition process. The same signalization process is utilized for the regulation of various vital functions by hormones. One of the well-known and typical neurotransmitters is catecholamine, such as dopamine and adrenaline etc., which has catechol ring and amine chain. Investigation of the conformational geometries of these molecules is important as fundamental knowledge to understanding the molecular recognition process of the neurotransmitters. In this work, we applied laser desorption supersonic jet laser spectroscopy, which enables the gas phase spectroscopy of biomolecules with distinguishing each conformers as isomers, to catecholamines and the irrelevant molecules. From the results, it was found that catecholamines have specifically small number, 1 or 2, of conformers in gas phase, while the relevant molecules which do not have catechol ring have many conformers, more than 10. This drastic reduction of number of conformers observed in catecholamines is attributed to neighboring phenolic OH group of catechol ring.

In addition, we turned our eyes to another lead role, receptor protein of catecholamines. However, since the receptors are huge molecules, whose molecular weights are larger than 40,000, it will be unreal to apply the gas phase spectroscopy to themselves. Fortunately, it was suggested that the binding site of the catecholamines is a small number of residues, so we projected the gasphase spectroscopy of the synthesized partial peptide of the binding site. We took notice of 5-recidue sequence of adrenergic beta 2-receptor, Ser-Ile-Val-Ser-Phe, which behaves as the binding site of catecholic OHs, and applied the laser desorption supersonic jet spectroscopy. By measuring IR spectrum and comparing it with results of quantum chemical calculations, we determined a geometrical structure of the 5-recidue peptide. For the next step, we are planning to generate a complex of 5-residue peptide and adrenaline in gas phase and determine its structure by IR spectroscopy and theoretical calculations.

Oral Session

Monday, 17th September — Room B-1

09:00 – 11:00

Session 3: Structures and Dynamics of Atomic and Molecular Clusters

Chair: Fuminori Misaizu (Tohoku University, Japan)

S03-1000
10:00 – 10:20

The 'sticky business' of cleaning gas-phase membrane proteins: a detergent oriented perspective

Antoni J Borysik, Carol V Robinson
University of Oxford

Keywords:
ClustersMSMSion-mobilityCIDdetergents

Novel aspects:
Detergent cluster characterisation using ion mobility mass spectrometry. Mechanism of cluster dissociation

Abstract:
Recent advances in the field of biological mass spectrometry have shown that it is possible to use this technique to obtain crucial insights into the subunit stoichiometry, topology and lipid binding of membrane protein complexes. Central to this approach is the use of collision induced dissociation (CID) to remove protein-bound detergents prior to mass analysis. However, this 'clean-up' strategy employs brute force to thermally agitate the ions to levels than can often result in the disruption of protein structure, thus abrogating any downstream structural characterisation. In order to improve these strategies there now exists a need to understand the mechanism/s by which detergent clusters protect protein complexes during CID. This may then lead to more rational selection criteria for detergents that are best suited for the gas-phase characterisation of membrane protein complexes.
In order to understand these processes we have characterised a range of different detergent clusters using electrospray ionisation mass spectrometry (ESI-MS) in tandem with ion mobility mass spectrometry (IMS) and tandem mass spectrometry (MSMS) . These important initial studies have been conducted in the absence of proteins which tend to occlude all of the detailed information that can be gained when studying the cluster in isolation.
Aside from having packing densities to within a few percent of micelles in solution we show that detergent clusters are unique to the gas-phase. Detergent clusters have topological features that are not representative of micelles in solution. Furthermore, the properties of these clusters do not depend on amphiphile orientation prior to the electrospray process. We also show that the well-known relationship between aggregate size and alkyl chain length in solution is actually reversed in the gas-phase. Thus, our data suggest that detergent clusters are formed non-specifically during the ESI process. The distribution of different aggregate sizes and shapes favours a system explained by weak forces governed by the cluster surface tensions.
In order to deduce the mechanism by which detergent clusters protect membrane proteins during CID we then investigated the properties of the clusters during thermal agitation by MSMS. We show that detergent clusters dissociate by mechanisms that are predominately described by charge fission processes and the loss of neutrals by evaporation. The latter of these two processes can be extensive and probably explains the high persistence of residual clusters even at maximum CID thresholds. Using these data we propose that evaporative cooling mechanisms protect membrane protein complexes during CID. Future directions designed to confirm this proposal are also discussed.

Oral Session

Monday, 17th September

Room B-1

09:00 – 11:00

Session 3: Structures and Dynamics of Atomic and Molecular Clusters

Chair: Fuminori Misaizu (Tohoku University, Japan)

S03-1020

10:20 – 10:40

Adsorption, Activation and Reaction of Molecules on Size-Selected Metal Cluster Ions

Masahiko Ichihashi[1], Shinichi Hirabayashi[2]

[1]Toyota Technological Institute, Ichikawa, Japan, [2]Genesis Research Institute, Inc. Ichikawa, Japan

Keywords:
copper clusters, CO oxidation

Novel aspects:
A carbon monoxide molecule reacts with an oxygen atom on copper cluster anions. This CO oxidation proceeds efficiently on copper pentamer and nonamer anions.

Abstract:
In a chemical reaction of molecules on a metal cluster composed of several to tens of atoms, its constituent metal atoms contribute cooperatively in some cases to the chemical reaction. The electronic and the geometric structures change sensitively in a reaction pathway, and the fundamental features of the reaction pathway vary characteristically with cluster size. One of our goals is to elucidate the relation of the reactivity with the electronic and geometric structures. This relation will provide a recipe for developing catalysts of a high utility. To this end, we studied reactions of molecules on metal cluster ions by use of a tandem mass-spectrometer with reaction cells. For instance, we investigated the reaction of CO and O_2 on size-selected copper cluster ions, Cu_n^+ (n = 4-18) and Cu_n^- (n = 4-11). A coadsorbing product, $Cu_nO_2(CO)^+$, was observed in the reaction of $Cu_nO_2^+$ with CO, and it was found that CO adsorbs onto $Cu_nO_2^+$ more efficiently than onto Cu_n^+. This shows that CO adsorbs cooperatively with O_2 onto Cu_n^+. On the other hand, in the reaction of $Cu_nO_2^-$ with CO, a reduced product, Cu_nO^-, was given instead of the coadsorbing product, $Cu_nO_2(CO)^-$. In particular, $Cu_5O_2^-$ and $Cu_9O_2^-$ have relatively high efficiency for the production of Cu_nO^-. This result suggests that CO is oxidized and CO_2 is produced on Cu_n^-. Further, we found that $Cu_5O_4^-$ is reduced sequentially and $Cu_5O_m^-$ (m = 0-3) are observed in higher CO pressures.
The DFT calculation indicates that the activation energy in the reaction of $Cu_5O_2(CO)^-$ -> $Cu_5O(CO_2)^-$ is only 0.79 eV while that of the corresponding cation is 1.79 eV. The structure of Cu_n^- is more flexible than that of Cu_n^+ probably because of its excess electron. It is concluded that the stabilization of the transition state and the decrease of the activation energy make the CO oxidation proceed on Cu_n^-.

Oral Session

Monday, 17th September

Room B-1

09:00 – 11:00

Session 3: Structures and Dynamics of Atomic and Molecular Clusters

Chair: Fuminori Misaizu (Tohoku University, Japan)

S03-1040
10:40 – 11:00

Formation, Characterization and Reactivity of a Gaseous Adduct of Carbon Dioxide to Magnesium(I), HOMgCO2-

Héloïse Dossmann (Soldi-Lose)[2], Carlos Afonso[3], Denis Lesage[2], Jean-Claude Tabet[2], Einar Uggerud[1]

[1]University of Oslo, [2]Pierre and Marie Curie University (Paris VI), [3]University of Rouen

Keywords:
Carbon dioxide fixation · gas phase reactions · computational chemistry · nucleophilic substitution · photosynthesis

Novel aspects:
We report a key intermediate of high relevance to natural and synthetic activation and fixation of carbon dioxide.

Abstract:
Activated forms of carbon dioxide are of great interest due to the relevance to photosynthetic CO_2 uptake and to sequestration of CO_2 from flue gases formed upon the burning of fossil fuels. One efficient fixation of carbondioxide by covalent C-C bond formation is schematically described as RX +CO_2 + 2 e^-® RCO_2^- + X^-. In plant cells, electrons are provided from chlorophyll via NADPH and formation of a C-C bond between CO_2 and another substrate occurs at the magnesium-centered active site of the enzyme RuBisCO. In this context, we report here on the gas-phase characterization of a novel and fragile structural form of carbondioxide bonded to magnesium (I) , $HOMgCO_2^-$ showing interesting reactivity features

$HOMgCO_2^-$ ions were formed via electrospray ionization of a mixture of oxalic acid and magnesium salts $MgCl_2$ and $MgBr_2$dissolved in CH_3OH/H_2O (v/v 90/10) . Analysis of the ions was done using a homemade modified Quattro II (Micromass, Manchester, U.K) and a hybrid quadrupole Fourier transform ion cyclotron resonance (hQh-FT/ICR, Solarix, Bruker Daltonics, Billerica, USA) mass spectrometer. Different types of activation were performed on the complexes, including collision-induced dissociation (CID) , sustained off-resonanceirradiation collision-induced dissociations (SORI-CID) and ion-molecule reactions (with H_2O and CH_3Cl) .
In parallel to experiments, quantum chemical calculations were carried out using the program system GAUSSIAN 09. Geometry optimization and single point energies were obtained using the composite G 4 method.

Electrospray ionization was applied to mixtures of oxalic acid and magnesium salts $MgCl_2$ and $MgBr_2$dissolved in CH_3OH/H_2O (v/v 90/10) . From the recorded mass spectra, signal corresponding to the $MgC_2O_5H^-$ion was observed. Upon collisional activation, this ion dissociates by losing CO_2, 2 CO_2 and MgC_2O_4 leading to the formation respectively of $HOMgCO_2^-$, $HOMg^-$and HO^-. Different structures are probed theoretically for the ion of interest, $HOMgCO_2^-$ as well as its mechanism of formation from the $MgC_2O_5H^-$ ion. Results show that two structures are conceivable for this ion, HOMg (η^2-O_2C)$^-$and HOMg (η^2-OCO)$^-$, separated from each other by 31kJ/mol. Furthermore, the complex shows a strong reactivity upon water which is present as a trace in the mass spectrometer. This reaction leads to the formation of an unique ion, $Mg(OH)_3^-$. Theoretical study of the mechanism of formation of this ion reveals a very exothermic pathway from HOMg (η^2-O_2C)$^-$ but not from HOMg (η^2-OCO)$^-$.As will be discussed, this supports the hypothesis of the presence of only one structure for $HOMgCO_2^-$, i.e. HOMg (η^2-O_2C)$^-$.Finally, the reaction of the complex with CH_3Cl is investigated. Several product ions are observed, in particular Cl^- and $CH_3CO_2^-$that are products from an S_N2-type reaction.

Oral Session

Monday, 17th September

Room D

09:00 – 11:00

Session 4: Imaging-I

Chair: Mitsutoshi Setou (Hamamatsu University School of Medicine, Japan)

S04-0900

09:00 – 09:40

[Keynote Lecture] Imaging molecular signals with multimodal imaging mass spectrometry

Ron M Heeren

FOM-Institute AMOLF

Keywords:
Imaging mass spectrometry, high resolution, microscope mode, detector technology, quantitative proteomics

Novel aspects:
A new multimodal MS imaging approach reveals changing signaling pathways in disease

Abstract:
The study of molecular signaling processes related to diseases requires not only the detection and analysis of the molecules involved but also the evaluation of their spatial organization. This allows the detailed chemotyping of cells under duress and can provide critical insight in the activated pathways related to the progression of the disease. Imaging mass spectrometry is ideally suited for this task as it allows the generation of high resolution molecular images of a wide variety of biological compounds from complex surfaces without the need for chemical labels or antibodies. High fidelity molecular ion images can be generated using modern desorption and ionization techniques combined with high end mass spectrometric systems. The distribution of several hundreds of peptides and proteins on the surface of histological tissue sections can be determined in a single imaging MS experiment. This enables molecular pathway analysis and the role of the different molecular signals and their behavior under a drastically changing chemical or biological environment in these pathways.
The complexity of these pathways in diseases prohibits the detailed chemical characterization by a single technique. We have developed an approach that combines a number of innovative mass spectrometric imaging approaches with bright field imaging, magnetic resonance imaging and targeted IHC images to refine our understanding of disease progression in human tissue samples. In addition we have integrated state-of-the-art quantitative proteomics and metabolomic studies of selected tissues in our molecular imaging workflow. The common histological tools typically only provide generic morphological information unless immunohistochemistry is used to determine the distribution one specific known protein. Imaging mass spectrometry has evolved to bring together these two disciplines, mass spectrometry and histology. This approach, sometimes referred to as molecular histology, can take great benefit from the use of high resolution mass spectrometry, gas-phase ion mobility separation and smart surface preparation protocols. Combined they provide new molecular visualization tools for biomedical researchers. These innovative developments towards high resolution imaging mass spectrometry can reveal new tissue details that remain hidden with conventional molecular imaging approaches. In this contribution we will discuss applications of new chemical microscopes in biomedical tissue analysis. This lecture will discuss how imaging MS can elucidate the way in which local environments can influence signaling pathways in diseases.

Oral Session

Monday, 17th September

Room D

09:00 – 11:00

Session 4: Imaging-I

Chair: Mitsutoshi Setou (Hamamatsu University School of Medicine, Japan)

S04-0940 Single cell level mass spectrometry imaging for plant metabolites

09:40 – 10:00

Young-Jin Lee[1,2]

[1]Iowa State University, Ames, USA/Iowa, [2]Ames Laboratory-USDOE, Ames, USA/Iowa

Keywords:
MALDI, mass spectrometry imaging, plant, metabolites, single cell

Novel aspects:
single cell level imaging on plant metabolites ; multiplex MS imaging technology to save data acquisition time while achieving rich chemical information.

Abstract:
We develop mass spectrometric (MS) imaging techniques to map metabolite distributions within plant tissues, particularly in single cell level high spatial resolutions. In this presentation, I will present a few of our recent work that demonstrate some technological advancements and applications to plant metabolic biology.

Technology development 1-Single cell level MS imaging of a whole flower : Chemical images of epiculticular wax lipids were acquired on the surface of Arabidopsis flower in 12um ultrahigh spatial resolution. Optical fiber with 25um core diameter was used to narrow the laser beam size and modified oscillating capillary nebulizer was used to homogeneously spray colloidal silver as a MALDI matrix. With this approach, single cell level very fine chemical images could be obtained including visualization of single pollen grains and fine structures of anthers.

Technology development 2-Multiplex MS imaging : Multiplex MS imaging technology has been developed to reduce the data acquisition time while obtaining diverse chemical information in a single experiment. In this methodology, each raster step is split into a few spiral steps. While orbitrap mass spectrometer is scanning dataset from the first spiral position, ion trap is scanning next few dataset from the following few spiral steps. Using this approach, we could simultaneously obtain high mass resolution imaging in low spatial resolution and high-spatial resolution imaging in low mass resolution, in much less data acquisition time. In addition, we could also incorporate a few MS/MS and MSn imaging at the same. We successfully applied this technique to obtain asymmetric distribution of structural isomers of flavonoids. This methodology is also applied to latent finger printing, simultaneously obtaining MS imaging and MS/MS imaging, and further extended to obtain positive and negative ion MS imaging in a single experiment.

Application 1-MS imaging as a tool for high-resolution functional genomics : Metabolite images from two genetically mutated Arabidopsis plants were studied using MS imaging and compared to those of wild-type. In MS imaging of CER1 mutant, systematic decrease of alkane, ketone, and C26 fatty acids were monitored on various plant tissues including flower, leaf, and stem, in contrast to slight increase of C30 fatty acids. High resolution MS imaging could distinguish C28 aldehyde from C29 alkane and revealed the accumulation of aldehyde in CER1 mutant, suggesting the involvement of CER1 gene in decarbonylation of aldehyde. Flavonoid imaging of the Arabidopsis flower showed an asymmetric distribution of kaempferol and its glycosides on the distal region of petals, and quercetin, isohamnetin, and their glycosides on the proximal region of petals. In contrast, a homogeneous distribution of kaempferol and its glycosides is observed in the mutant (tt7), suggesting asymmetric gene expression of TT7.

Application 2-Cellular level lipid distribution in cottonseeds : MS imaging of cryosections of cotton embryos revealed a distinct, heterogeneous distribution of molecular species of triacylglycerols (TAG) and phosphatidylcholines (PC), the major storage and membrane lipid classes in cotton embryos. Other lipids were imaged, including phosphatidylethanolamines, phosphatidic acids, sterols, and gossypol, indicating the broad range of metabolites and applications for this chemical visualization approach. Comprehensive lipidomics images generated by MALDI-MSI report accurate, relative amounts of lipid species in plant tissues and reveal previously unseen differences in spatial distributions providing for a new level of understanding in cellular biochemistry. For example, TAG species with palmitic and linoleic acids (c.f., TAG 50 : 2 (PLL) or TAG 52 : 4 (LLL)) have a higher abundance in cotyledon than in the embryonic axis. The similar distribution in PC (c.f., PC 34 : 2 (PL) or PC 36 : 4 (LL)) supports the synthesis of TAG via membrane lipids as an intermediate.

Oral Session

Monday, 17th September

Room D

09:00 – 11:00

Session 4: Imaging-I

Chair: Mitsutoshi Setou (Hamamatsu University School of Medicine, Japan)

S04-1000

10:00 – 10:20

Molecular Imaging of Cells and Tissues with Continuous Cluster Ion Beams

Jiro Matsuo[1,2], Takaaki Aoki[1,2], Toshio Seki[1,2]

[1]Kyoto University, Kyoto, Japan, [2]JST, CREST

Keywords:
Mass Imaging, SIMS, Lipid, Cluster ions

Novel aspects:
Advanced mass imaging system employing with Ar cluster ion beams. Mass images of cells and tissues will be presented.

Abstract:
Various mass spectroscopic techniques, such as secondary ion mass spectrometry (SIMS), matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI), have been examined intensively during the last decade, because molecular, structural and chemical state information is considered invaluable in life science. The SIMS technique is considered to have the highest spatial resolution, and submicron resolution has been reported for elemental analysis of inorganic materials. Energetic ion irradiation also leads to emission of molecular ions representing the chemical composition of biological materials. However, secondary molecular ion yields are usually quite low, because of decomposition of molecules and low-density excitation of surface. Novel ion beams, such as cluster ion beams and swift heavy ion beams, are utilized to enhance secondary molecular yields. Cluster ion beams have been reported to enhance the yields of secondary ions, because of the high-density energy deposition and multiple collisions near surfaces. SF_5, C_{60}, Au_3 and Bi_3 were found to be quite useful for SIMS of organic materials [1]. Because these primary ion beams cause significant damage on organic surfaces, the primary ion dose is limited to a certain threshold value (known as "the static-limit", ~10^{12} ions/cm^2). Therefore, the intensity of small molecular ions (>500 Da) is too low to obtain high-resolution mass images.
We proposed the use of a large Ar cluster beam for SIMS analysis of organic materials. This beam presents a number of advantages over other cluster beams for molecular depth profiling of various organic materials, and provides new opportunities for sputtering molecules without inducing significant damage [2, 3]. Biomolecules are also very fragile and thus difficult to sputter with conventional ion beams; therefore, a large Ar cluster beam would be quite suitable for biological material analysis. We have developed a new Ar cluster ion gun aiming to obtain a fine-focused beam, and Ar cluster ion beam with several mm diameters was obtained.
The beam current density is too low to measure mass spectra with the conventional time-of-flight (TOF) method, when the primary ion beam is pulsed. An orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer, which allows the use of a continuous beam, was employed in a new bioimaging system. There was no need to use the ion-bunching technique in this system, and therefore there was no need for tradeoff between beam diameter and mass resolution, which is a problem in mass-imaging of biological samples with conventional SIMS. This is another advantage of our new molecular imaging system over the conventional TOF instrument. Because of the complexity of chemical compositions in natural samples, such as cells and tissues, it is very important to maintain high mass resolution (>10000) to separate between different molecules with similar mass.
The latest results of this system and its performance in molecular imaging of cells and tissues will be presented and discussed.
Acknowledgements
This work is supported by the Core Research of Evolutional Science and Technology (CREST) of Japan Science and Technology Agency (JST).

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Oral Session

Monday, 17th September
09:00 – 11:00
Room D

Session 4: Imaging-I

Chair: Mitsutoshi Setou (Hamamatsu University School of Medicine, Japan)

S04-1020
10:20 – 10:40

Spatial Resolution of Metabolites during Barley Grain Development by Imaging Mass Spectrometric Analysis

Manuela Peukert[1], Andrea Matros[1], Ales Svatos[2], Hans-Peter Mock[1]
[1]IPK Gatersleben, Germany, [2]MPI Jena, Germany

Keywords:
MALDI MSI, metabolites, barley grain

Novel aspects:
Application of MALDI MSI to plant tissues such as grains for elucidation of metabolite distributions during development and direct identification of compounds from the tissue section

Abstract:
Spatially resolved analysis of metabolites and proteins is essential for elucidating compartmentalized cellular processes. During the last decade enormous progress has been achieved in MALDI MS based imaging (MALDI MSI) and application of this technique to animal and human tissues has revealed a number of novel clinical markers for better diagnosis or for pharmacological studies. Investigation of plant tissues with MALDI MSI is more recent and adaption of protocols is needed as plant organs comprise complex tissue types. For example, visualization of specific classes of compounds relies on the right conditions for ionization. We are interested in the elucidation of components involved in barley grain development. An understanding of developmentally and ecologically regulated processes affecting agronomical traits such as final grain weight, seed quality and stress tolerance is of outmost importance, as barley provides one of the staple foods.
The presentation will introduce an untargeted MALDI MSI approach to the analysis of metabolite patterns during barley grain development. We analyzed longitudinal and cross sections from developing barley grains (3, 7, 10 and 14 days after pollination). A number of candidate *m/z* values showing high tissue as well as developmental specificity were detected and selected examples will be shown. Challenging aspects regarding spatial resolution, sensitivity and identification of unknown compounds will also be discussed. First analytical efforts allowed for assignment of a number of the candidate *m/z* values to various metabolite classes, such as sugars, phospholipids and chlorophyll, directly from tissue sections. However, identification from tissue sections failed for many of the candidate *m/z* values due to their low abundance in the individual MALDI MS spectra. Thus, current work includes fractionated extraction of differentially distributed compounds out of manually dissected barley grain tissues and identification of respective molecular ions by GC-MS and LC-MS approaches. Finally, further approaches for quantification of selected metabolites by LC-MS and GC-MS as well as approaches for relative quantification of selected metabolites by DESI-MS will be presented. The future task will be to relate the patterns to distinct cellular and physiological events. First results revealed particular metabolite distributions indicative for nutrient transport into the developing endosperm and for grain hardness (e.g. dough and germination properties).

S04-1040

10:40 – 11:00

Mass spectrometry imaging (MALDI-TOF/TOF) of drugs in a brain mouse model of Parkinson disease

David Touboul[1], Hanane Kadar[1], Gael Le Douaron[2], Majid Amar[3], Bruno Figadère[2], Laurent Ferrié[2], Rita Raisman-Vozani[3], Alain Brunelle[1]

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Keywords:
mass spectrometry imaging, Parkinson disease

Novel aspects:
Drugs localization and lipid composition of brain from mice models of Parkinson disease

Abstract:
Introduction
Neurodegenerative disorders, such as Parkinson and Alzheimer diseases, constitute a major public health issue affecting more than thirty millions of people worldwide. Patients affected by Parkinson disease suffer from different symptoms, motor impairments as muscular rigidity, slowness of movement, trembling at rest. Although the etiology of the disease is not clearly understood, death of dopaminergicneurons in substancia nigra pars compacta (SNpc) has been found to be one of the main features of the disease. Therapies correcting the loss of dopamine only allow a decrease of some of the symptoms, but don't alter the neurodegenerative process. The goal of our experiments is to localize the drug (1-methyl-4-phenylpyridinium, MPP+) inducing the Parkinsonism in mouse model and to survey the modifications of lipid profiles in the different parts of the brain by MALDI-TOF/TOF imaging. A new synthetic active molecule (SF41), providing both neuroprotective and neuritogenic activities for dopaminergicneurons, was also localized by mass spectrometry imaging (MSI).
Methods
Experiments were performed using a MALDI-TOF/TOF (4800 and 5800 AB-Sciex) instrument. For each mouse brain (control, treated by MPTP or SF41), images of 5 sections at different depths were recorded. A major advantage of this technique is the opportunity to obtain a chemical mapping of a wide range of substances in a single run, particularly lipids. Then 9-aminoacridine andalpha-cyano-4-hydroxycinnamic acid matrices were deposited on the tissue sections with a TM-Sprayer for negative and positive modes, respectively. All instrumental parameters were optimized for the best sensitivity. Lateral resolution was fixed to 70 µm.
Results
MPP+ was detected in the positive ion mode in mouse brain treated by MPTP, which is the precursor of the active drug. Clear accumulations in the SNpc and the striatum, which represent the two areas rich in dopaminergic neurons and fiber, were demonstrated. Lipid profiles in both positive and negative ion modes were compared between control and MPTP-treated mouse. The process consists in the comparison of mass spectra of the relevant regions of interest (ROIs) so as to discriminate differences in lipid composition and to highlight some potential biomarkers for Parkinson disease. Significant modifications will be discussed.
SF41 was also detected in the positive ion mode in treated mouse brain. This indicates that SF41efficiently crosses the blood-brain barrier as confirmed by LC-MS/MS. SF41 was detected in different anatomical brain areas, especially the striatum and the SNpc. Ion images were associated to immunohistochemistry TH+ and Nissl preparation of adjacent brain sections to precisely localize the altered areas. It emphasizes that the compound reaches its target and can have an *in vivo* neuroprotective effect on dopaminergic neurons loss. EasyMSI processing software was used for this purpose. Lipid profiles were also extracted from the MSI data and compared to the ones from the control or MPTP-treated mouse brain.

Oral Session

Monday, 17th September

Room E

09:00 – 11:00

Session 5: Advances in Spray Ionization Techniques

Chair: Charles N McEwen (University of the Sciences, USA)

S05-0900 [Keynote Lecture] Cold-Spray Ionization Mass Spectrometry

09:00 – 09:40

Kentaro Yamaguchi

Tokushima Bunri University, Kagawa, Japan

Keywords:
CSI-MS, non-covalent-complex, charged probe

Novel aspects:
Cold-spray ionization method was presented. Application to labile organic species as well as non-covalent complexes are shown. Metal-complex-based ionization probes exhibited effective ionization by using cold-spray ionization method.

Abstract:
Electrospray ionization (ESI) MS is generally used for the characterization of labile supramolecules in which non-covalent bonding interactions are predominant. However, molecular ions are not detected in many cases because of their instability, and even if the molecular ions are detected, thermal decomposition generates fragment ions whose peaks also appear in the mass spectrum. In conventional ESI, the heat produced by the desolvation chamber was thought to be necessary for the ionization in the gas phase ; however, it might be responsible for the thermal decomposition.

Cold-spray ionization (CSI) is designed for the MS detection of labile organic species. It may be an appropriate method to analyze the structures of biomolecular complexes and labile organic species in solution. The method, a variant of ESI-MS, operates at low temperatures and can simply and precisely characterize labile non-covalent complexes that are difficult or impossible to observe by conventional MS.

CSI is particularly suitable for elucidating the structures of labile organometallic compounds in solution. This method provides a means to investigate the dynamic behavior of unstable molecules and/or labile clusters in solution.In the first example, the composition of Grignard reagent in solution was investigated. CSI-MS revealed that $RMg_2(m\text{-}Cl_3)$ is the structure of RMgCl in THF solution. CSI-MS is also a powerful tool to analyze the equilibria of multiply linked self-assembling catenanes in solution. Its application to unstable and complex supramolecules will be shown.

The CSI-MS can be used to investigate labile solution structures of various biomolecules. In the case of DNA, for example, various oligodeoxynucleotide complexes can be observed by CSI-MS ; examples include such unstable species as low *Tm* DNA duplexes. CSI shows its superiority to other methods by effectively elucidating the interactions of DNA complexes. In the case of amino acid analysis, aggregation to form corresponding clusters is widely observed. Large-scale aggregated chain structures of simple biomolecules, such as amino acids, can be introduced to the gas phase. A clear chain structure can be seen for amino acids, including glycine, L-valine, L-serine, L-methionine, and L-phenylalanine, by means of CSI-MS. A difference, diffusion-based NMR technique and CSI-MS can be employed as a solution-based approach to identify a ligand binding to a protein receptor. The difference, diffusion-based NMR technique, called difference NOE pumping, can directly detect a ligand binding to a protein receptor. The CSI method can also detect the ligand-receptor complex. The efficiency of these techniques for identifying binding ligands is demonstrated with a human serum albumin HAS-drug system.

Various labile organic compounds are analyzed by the CSI method in organic chemistry. Investigations of real solution behavior of labile organic molecules by MS are difficult. The nature of hydrogen-bonding interactions remains unclear even for simple water molecules, to say nothing of the precise behavior of complex biomolecules in solution. This is because higher-order structures are linked by weak intermolecular interactions that are readily disrupted. CSI-MS was used to investigate the solution behavior of aggregated steroid compounds, bisguanidinobenzene-benzoicacid complexes, and cavitand cages.

Recently, we developed an effective ionization method that uses metal-complex-based ionization probes containing 2,6-bis (oxazolinyl) pyridine (pybox) ligands. By using these probes, we were able to obtain multiply charged ions of target molecules. This method effectively ionized large complex molecules, including biomolecules and various supramolecules, as well as carbon clusters, such as fullerenes. Moreover, isotope-labeled pybox-La complexes were used to clearly detect isotopic labeling shifts. Their applications to multiply charged ionization, including isotope labeling of biomolecules and carbon clusters using CSI-MS, will be shown.

S05-0940

09:40 – 10:00

Mass Spectrometric Analyses of Isomeric Sugars, Lipids and their Conjugates: Introduction to "Charge-Localization Isomers"

Yoko Ohashi[1,2], Masayuki Kubota[3], Takashi Hirano[2]

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Keywords:
charge-localization isomers ; ESI-MS/MS ; glycosaminoglycan

Novel aspects:
ESI-MS/MS is not only capable of distinguishing conventional isomers but also has shown existence of a new class of isomers.

Abstract:
With an unexpected success in distinguishing positional isomers of phospholipids in the early '80s, we used FAB-MS for isomer distinctions of sphingoid isomers, ganglioside series and sugar linkages. Afterwards, ESI-MS was found to be more promising in accuracy with much less sample consumption. Recently, we have come to think that we have covered isomer distinctions of essentially all categories of similar biological isomers by mass spectrometry when stereoisomers such as epimers and anomers were finally successfully distinguished by ESI-MS/MS. [1, 2]

However, an extensive examination of an unsolved problem left in the elucidation of one minor fragmentation path has led us to conclude that there is a new class of isomers, which we call "charge-localization isomers ". This specific class of isomers may exist transiently as the negative ion of strong dibasic acids, exemplified here as a glycosaminoglycan disaccharide *N*-acetyllactosamine-6, 6'-disulfate disodium salt (M"W 587, where M" stands for the disodium salt) . [3] As shown in ref. 3, a negative precursor ion $[M"-Na]^-$ at *m/z* 564, originating from this dibasic acid, is capable of fragmenting by two different routes in negative ion ESI-MS/MS. Two possibilities ought to be considered : (1) There is only one common precursor ion for the two routes. (2) There are two different precursor ions individually leading to two different fragmentation routes. In the former case, such precursor ion structure may represent a doubly dissociated negative ion weakly bridged by a Na^+, which compensates one negative charge. This situation will be conceivable in the solution phase. However, at the spray ionization, Na^+ must localize at either one of the two sulfate anions in the gas phase, eventually leading to the latter case. Evidence is provided as follows.[3] The negative ion ESI-MS full scan spectrum of *N*-acetyllactosamine-6, 6'-disulfate disodium salt showed a doubly negative ion $[M" - 2\,Na]^{2-}$ at *m/z* 270.5 in higher abundance than $[M"-Na]^-$ at *m/z* 564. This means that the two negative groups are well separated owing to the Coulomb repulsion. Since a preliminary optimization of the structure of the dihydrogensulfate by semi-empirical molecular orbital calculations (PM5) indicated that the two hydrogen sulfate groups were geometrically far apart,[1] the mechanism including two different precursor ions is more probable. Molecular structure optimization by DFT calculations is now in progress.

We assume that either one of the two sulfate groups is available for the negative center. Thus, the two formed individual negative ions (charge-localization isomers) proceed along different routes of decomposition, even though some product ions apparently show isobaric mass numbers. Thus, we claim that the entity of $[M"-Na]^-$ is not a Na^+-bridged doubly negative ion but a mixture of two separate singly negative ions, which we call "charge-localization isomers ".

Experimental :
Galb1-4GlcNAc-6,6'-disulfate disodium salt (M"W 587) was obtained from shark fin keratan sulfate by keratanase II digestion, and dissolved in H_2O/CH_3OH (1 : 1) at a concentration of 10 ng/mL. A ThermoFisher Scientific mass spectrometer LTQ FT was used in the accurate mass measurement mode with the spray voltage 1 kV and the resolving power 100,000. The CID energy parameter for MS^2 and MS^3 was 30%.

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Oral Session

Monday, 17th September

Room E

09:00 – 11:00

Session 5: Advances in Spray Ionization Techniques

Chair: Charles N McEwen (University of the Sciences, USA)

S05-1000

10:00 – 10:20

"Supercharging" and "Subcharging" Proteins and Protein Complexes to Explore Electrospray Ionization

Rachel R Loo, Rajeswari Lakshmanan, Jiang Zhang, Joseph A Loo

University of California-Los Angeles, Los Angeles, CA/USA

Keywords:
supercharging, conformation, charge state distributions, charged residue model, electrospray mechanism

Novel aspects:
The role of various solution additives in altering charge distributions forms a basis to explore the mechanism of electrospray ionization.

Abstract:

Introduction

Increasing charge deposited on analytes by electrospray ionization is desirable, because higher charged molecules are more effectively dissociated by tandem MS and because they reduce analyzer *m/z* requirements. Supercharging ability is especially valuable when examining protein-protein and protein-ligand associations where, for many non-covalent complexes, a sufficiently broad reagent concentration window exists to elevate charge without altering stoichiometry ; *e.g.*, with the 28 subunit, 600-kDa 20S proteasome and with 14 subunit, 800-kDa GroEL. Regardless of supercharging's utility, the mechanism responsible for that charge increase is under debate, as is the question of whether separate mechanisms are required to describe the charge elevation observed from non-denaturing and denaturing solutions. Here we consider experimental observations of protein charging with regard to an ESI mechanism.

Methods

Positive ion electrospray ionization mass spectra were acquired with three different systems : a hybrid quadrupole time-of-flight (QTOF) mass spectrometer with Triwave ion mobility (IM) separator (QTOF/IM ; Waters Synapt HDMS, Manchester, UK) , an LTQ-FT Ultra mass spectrometer (Thermo Fisher Scientific) , and an LTQ ion trap mass spectrometer (Thermo Fisher) . The nanoESI source using borosilicate glass capillaries with Au/Pd coatings (Proxeon Biosystems, Odense, Denmark) was operated at low analyte flow conditions (50 nL/min) . Protein samples were desalted with centrifugal filter devices using 20 mM ammonium acetate prior to analysis. The final protein concentration for ESI-MS measurements from non-denaturing solutions was approximately 5 uM in 20 mM ammonium acetate, pH 6.8. Reagents for altering charge included *m*-NBA, sulfolane, DMSO, hexafluoroacetone, and others.

Results

Supercharging of denatured solutions has been suggested to arise from the increased surface tension imparted to late-stage ESI droplets by low-volatility additives. Since that proposal, complicated boiling point/evaporation arguments have sometimes been applied to rationalize or to question how surface tension would vary during an organic/water/acid-containing droplet's lifetime. We return to these considerations, and also to the charged residue model (CRM) , essential to justifying a relationship between charge and surface tension.

It is notable that proponents of the CRM assert that native proteins and compact molecules ionize by the classic CRM, whereas denatured proteins must instead be described by a modified CRM or ion evaporation. Modifications are needed to rationalize CRM's poor fit to denatured proteins' predicted and observed charge distributions. Modified CRM argues that non-spherical droplets deform without exploding, stabilized by the polymer backbone to attain charge densities beyond the limit imposed by the solvent surface tension. However, the need to invoke "stabilization " to rationalize the observed charge distributions of denatured proteins invalidates the Rayleigh limit relationship to surface tension. Notably, the results most often used to support surface tension dependence in supercharging are obtained from denaturing solutions.

Reagents increasing or decreasing charge in electrospray ionization for native and denaturing solutions, independent of conformational changes, can be divided into several classes and assessed for volatility, basicity, acidity, and ability to alter charge in positive and negative ionization modes. These classes provide hints about relevant considerations in electrospray ionization charge state distributions and to the mechanism of ionization.

Impact on charge state distributions of gas versus solution phase denaturation and gas versus solution phase charge transfer are also considered, as is the presence of supercharging reagent adducts that localize primarily to higher charge ions.

Oral Session

Monday, 17th September

Room E

09:00 – 11:00

Session 5: Advances in Spray Ionization Techniques

Chair: Charles N McEwen (University of the Sciences, USA)

S05-1020

10:20 – 10:40

Ionization Methods Requiring as little as the vacuum of the Mass Spectrometer to Produce Highly-Charged Ions from Surfaces or Solutions

Sarah Trimpin

Wayne State University, Detroit, MI

Keywords:
vacuum ionization, spray ionization, imaging, instrumentation, highly charged ions

Novel aspects:
Novel ionization methods at atmospheric pressure and vacuum not requiring voltage, laser, or heat to produce highly charged ions directly from native, complex samples such as tissue

Abstract:
We recently introduced a new mass spectrometric method applicable to proteins which uses laser ablation of a matrix-analyte mixture similar to atmospheric pressure **MALDI** but produces mass spectra nearly identical to **ESI**. This new technique called laserspray ionization (**LSI**) (***[1]***) has advantages of speed of analysis, high spatial resolution for imaging, mass range extension, and improved fragmentation common with multiply charged ions. This approach was extended to producing highly charged ions from proteins by laser ablation using vacuum MALDI sources (***[2]***) . We demonstrate that proteins as large as BSA (~66 kDa) produce ESI-like multiply charged ions from certain matrices when introduced to vacuum from AP. Crucial for the production of highly charged ions are desolvation of the matrix from charged matrix/analyte clusters by thermal or vacuum assistance and the use of a laser is just a sophisticated means of transferring the clusters into the gas-phase.
Applying this concept, we show first examples of highly charged ions produced directly from a solid matrix-analyte material using a vacuum MALDI source without need of a laser. Charge states up to 44 are observed for BSA. Protein, peptide, and lipid ions are observed directly from mouse brain tissue and analyzed using IMS and MS (SYNAPT G2) separations without the need of harsh conditions imposed by laser ablation. Initial results for protein complexes directly from surfaces will be shown. Other uses are rapid and automated analyses of small molecules such as drugs and body fluids including blood spots, saliva, and urine. Producing ions under vacuum conditions in a mass spectrometer enhances sensitivity and the multiply charged ions offer the potential to use high performance mass analyzers for advanced structural characterization. Such a simple ionization method, requiring only the vacuum necessary for the proper functioning of the mass spectrometer, should prove useful in clinical settings and in field portable instruments.
We show that all of the newly developed AP and vacuum methods as well as ESI produce ubiquitin ions of similar structures as determined by IMS-MS. Further, multiply charged ESI-like ions are also produced from solvent solutions allowing us to interface this method with a NanoAcquity UPLC for separation prior to high sensitivity mass analysis of, e.g., drugs and protein digests (***[3]***). The multiply charged ions from any of these methods produce nearly complete backbone fragmentation using electron transfer dissociation. Applications, besides high sensitivity LC/MS, include tissue imaging and characterization of fragile ganglioside lipids and neuropeptides directly from mouse brain tissue (***[4]***) .

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Oral Session

Monday, 17th September

Room E

09:00 – 11:00

Session 5: Advances in Spray Ionization Techniques

Chair: Charles N McEwen (University of the Sciences, USA)

S05-1040

10:40 – 11:00

Rapid Characterization of Chemicals in Plant via Direct Electrospray Probe Coupling with Mass Spectrometry

Hung Su, Chu-Nian Cheng, Min-Zong Huang, Jentaie Shiea

National Sun Yat-Sen Univrsity, Kaohsiung, Taiwan

Keywords:

Direct electrospray probe ; plant tissue ; Steviarebaudiana

Novel aspects:

Direct electrospray probe mass spectrometry was used to rapid characterization of chemicals in plant.

Abstract:

Electrospray ionization (ESI) , an atmospheric ionization method, can directly ionize analytes in liquid and provide an effective means of analyzing large biomolecules as well as small organic and inorganic compounds. In ESI, however, the sample solution subjected to ESI/MS analysis must be carefully cleaned to prevent from capillary clog. Direct electrospray probe (DEP) has been developed as a direct sampling ionization method for mass spectrometric analysis of complex solution without the need ofa sample introduction capillary. Ions of analyte are directly generated by applying a high voltage to the probe wetted with a small volume of solution. The DEP technique provides a number of unique analytical features including : (a) sample consumption is low and sample switching is quick ; (b) the capillary and pump are non-required ; and (c) the probe is low cost and easy to be constructed and cleaned. In this study, a modified DEP probe is successfully applied to characterize the chemicals from plant tissues (e.g. leaf, stem, and root) for disease diagnosis.

In DEP, the analyte molecules are first dissolved while the extract solvent is pre-loaded onto the plant tissues. Next, analyte ions are generated from the tip of probe via electrospray ionization. In this study, we demonstrated that the main components of plant tissues were successfully determined through the DEP analysis under ambient conditions. For example, both positive and negative ions of the organic compounds such as steviolbioside, rubusoside, stevioside, dulcoside A, and rebaudioside E in the plant tissues of *Steviarebaudiana* were detected directly via DEP technique. Furthermore, the distribution of organic compounds was obtained by using DEP to directly examine different areas of the same plant tissue. For example, different molecular ions were acquired from the green- and white-color surface of the *Erythrina indica* var. picta tissue. In consequence, we demonstrated that DEP technique can be applied to directly and rapidly characterize and identify major compounds in plant tissue and its great potential for the diagnosis of plant diseases.

Oral Session

Monday, 17th September

Main Hall

15:00 – 17:00

Session 6: Novel Approaches in Proteomics Analysis

Chair: Roman A Zubarev (Karolinska Institutet, Sweden)

S06-1500

15:00 – 15:40

[Keynote Lecture] Instant Spectral Assignment for Rapid, High-Throughput Targeted Proteomics

Derek J Bailey, Amelia C Peterson, Christopher M Rose, MIchael S Westphall, Joshua J Coon

University of Wisconsin-Madison/Madison, Wisconsin/USA

Keywords:
Selected Reaction Monitoring, Multiple Reaction Monitoring, Targeted Proteomics, Shotgun Proteomics, Orbitrap

Novel aspects:
Use of FT-MS for large-scale targeted proteomics

Abstract:
The most widespread technique in proteomics is the shotgun method. Proteins are digested into peptides, chromatographically separated, and measured by mass spectrometry (MS). Many types of mass spectrometers are used quadrupole ion traps (QIT), QIT hybrids such as the Orbitrap or LTQ-FT-ICR, and quadrupole time-of-flight (Q-TOF) but the experiments from MS measurements onward are basically the same. Eluting peptide cations have their mass-to-charge (*m/z*) values measured in the MS^1 scan. The most abundant precursors are then selected for a series of sequential tandem MSevents (MS^2). The number of these events depends on the acquisition rate of the device, but generally ranges from 3 to 15. Following a second MS^1 scan, a new group of targets is selected. The process, called data-dependent acquisition (DDA) continues for the duration of the chromatographic separation. Because this method has not changed over the past fifteen years, advancements have come from the constant evolution of the MS hardware. Significant improvements in key Figures of Merit such as sensitivity, scan rate, mass accuracy, and resolution have evolved over this period. Constant operation generates hundreds of thousands of spectra in days. These spectra are then mapped to peptide or protein sequence using highly evolved database search algorithms. Successful results can be obtained within just a few days of instrument analysis, and are nothing short of spectacular : tens of thousands of unique peptide spectral matches mapping to several thousand unique protein isoforms have become the norm.

Doubtless the shotgun approach achieves high throughput, it does, however lack sensitivity and reproducibility. Specifically, complete coverage of specific pathways or functional groups is not typical (*i.e.*, all 500 kinases, 1,400 transcription factors, *etc.*). Likewise, overlapping IDs in replicate experiments are low (35-60 %) **.** This lack of completeness and reproducibility limit the quantity and quality of biological conclusions that can be drawn from a proteomic experiment. These limitations have propelled recent fervor in target-based methods, namely selected reaction monitoring (SRM). SRM achieves the reproducibility that the shotgun approach lacks and can determine *absolute*abundance, but suffers from low throughput and low resolution, as it is primarily restricted to the triple-quadrupole (QqQ) MS platform. Here we describe a new data acquisition paradigm that harnesses the mass accuracy and speed of new generation of high resolution hybrid MS systems to develop to achieve the sensitivity and reproducibility of SRM methods with the high-throughput capacities of the shotgun technique. Our approach is to a new targeting concept, parallel reaction monitoring (PRM), that is uniquely enabled by the high-resolution mass analyzers. PRM stands to remedy the extremely low throughput problem of SRM by exploiting high resolving power to readily distinguish target product ions from contaminants. To test this, we analyzed a test-set of fourteen synthetic peptides, present in a biological matrix of yeast tryptic peptides, using either the PRM method (Q-Exactive) or traditional SRM (QqQ). These data demonstrated low-attomole level detection limits, high measurement reproducibility, and good linearity over 5 orders-of-magnitude for PRM. PRM analysis, on average, was significantly more sensitive than QqQ SRM, quantifying peptides over a greater number of concentration orders-of-magnitude (3.9 versus 3.2 average orders quantified, $p = 2.8E\text{-}3$, $n = 14$). The linearity of response between the two methods was the same (0.93 versus 0.87 average logged adjusted %RSDs, $p = 0.51$,$n = 14$). These data suggest that the PRM method, with very little assay development time (about 1 hr) can target analytes in matrix with the same or better fidelity than a highly optimized QqQ SRM method (extensively developed over several days).

Oral Session

Monday, 17th September

Main Hall

15:00 – 17:00

Session 6: Novel Approaches in Proteomics Analysis

Chair: Roman A Zubarev (Karolinska Institutet, Sweden)

S06-1540

15:40 – 16:00

Miniaturised Chemical Proteomics to Profile Clinically-relevant Kinase Inhibitors in Tumour Core Biopsies

Ivo Chamrad[2], Uwe Rix[3], Manuela Gridling[1], Katja Parapatics[1], Andre C Mueller[1], Alexey Stukalov[1], Giulio Superti-Furga[1], Eric B Haura[3], Keiryn L Bennett[1]

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Keywords:
kinase inhibitor, chemical proteomics, core biopsy, Orbitrap

Novel aspects:
The optimisation and miniaturisation of a robust method to study the kinase inhibitor profile of human tumour core biopsies

Abstract:
Specific anticancer agents have been undeniably successful in the treatment of the disease, however, several issues still remain. Even when enormous efforts have been made to design and develop compounds affecting only given molecular nodes in cancer circuits, strictly-selective agents are rare. The majority of novel, potential therapeutics display broader inhibitory profiles than originally envisaged. Affecting unexpected targets can lead to unwanted toxicity and cause adverse side effects. Alternatively, incidental drug promiscuity is not necessarily always harmful. Hence, for every drug, determining the specificity profile and delineating a plausible mechanism-of-action should be an integral part of the modern drug-discovery pipeline.
Chemical proteomics is a compound-centric affinity approach that utilises immobilised drugs to isolate protein interactors from protein mixtures. Identification of specific protein sets is achieved via modern high-end mass spectrometry (MS). Research in our laboratory has previously downscaled the affinity procedure to an input of 500µg total protein.[1] The study presented here, entailed a further miniaturisation of the protocol to 100µg - the range of material that can be obtained from a core biopsy. The possibility to perform systematic chemical proteomics with material from core biopsies taken from patient tumours will provide new opportunities for personalised medicine.
An analogue of the broad-spectrum tyrosine kinase-inhibitor bosutinib was coupled to sepharose beads.[2] Cell lysate was generated from the human K562 cell line (derived from chronic myelogenousleukaemia). Drug pulldowns were performed according to Fernbach *et al.*[1] except that the total quantity of input material was lowered to 100µg protein. Experimental conditions such as protein concentration, bead bed-volume, total quantity of drug, drug concentration on the beads, incubation time, agitation speed, elution volume, and quantity of trypsin were assessed as a multi-dimensional matrix. Samples were analysed byLCMS on hybrid LTQ Orbitrap instruments and based on the number of kinases identified, the experimental conditions were optimised for drug pulldowns from low quantities of protein.
In the first phase of this study, the published protocol[1] was assessed with 100µg protein input. Approximately 100 protein groups (98 and 120) were unambiguously identified in two replicates, with the target kinases accounting for ~20 % of all protein identifications. For specific interaction partners (~30 kinases), many known major targets of bosutinib such as SRC kinases (LYN and YES), Bcr-Abl, CSK, BTK, GAK, EPHB 4 and PTK 2 were present. Functional annotation analysis of the proteins revealed an over-representation of gene ontology terms associated with kinase activity. These results were highly-encouraging, as lowering the protein input by 5-fold still resulted in the identification of a high proportion of kinases. Only 10 fewer kinases were apparent as compared to the data obtained with 500µg protein input. Phase two of the study involved a systematic assessment of several experimental metrics that can influence the result of the chemical proteomic experiment. To monitor the various permutations of factors investigated in optimising the protocol, normalised sequence abundance factor (NSAF) was utilised. Several blocks of experimental parameters were designed and evaluated, prior to selecting the ideal conditions to design the next experiments. After several rounds, the following conditions were selected: protein concentration (0.5mg/mL); bead volume (50µL); total quantity of drug (50nmol); drug concentration (1 nmol/µL); incubation time (2 h); agitation speed (10r.p.m.); acid elution volume (250µL); and trypsin quantity (1.25µg). To ensure that our optimised approach was not tuned specifically for bosutinib, the down-scaled methodology will be assessed with additional tyrosine kinase inhibitors. Ultimately, the approach will be applied to lysates of core biopsies from tumour samples and the protein profile determined from clinically-relevant tyrosine kinase inhibitors.

[1] Fernbach, N., *et al.* (2009) *J Proteome Res* 8, 4753-4765
[2] Remsing Rix, L., *etal.* (2009) *Leukaemia* 23, 477-485

Oral Session

Monday, 17th September

Main Hall

15:00 – 17:00

Session 6: Novel Approaches in Proteomics Analysis

Chair: Roman A Zubarev (Karolinska Institutet, Sweden)

S06-1600

16:00 – 16:20

Combining ribosome profiling (ribosome captured mRNA sequencing) and mass spectrometry into an unprecedentedly comprehensive protein identification strategy.

Gerben Menschaert[1], Petra Van Damme[1,2], Jeroen Crappe[1], Geert Baggerman[3,4], Wim Van Criekinge[1]

[1]Ghent University, Ghent, Belgium, [2]VIB (Flemish Institute for Biotechnology), Ghent, Belgium, [3]Antwerp University CFP (Center For Proteomics), Antwerp, Belgium, [4]VITO, Mol, Belgium

Keywords:
ribosome profiling, COFRADIC, shotgun proteomics, alternative TIS

Novel aspects:
This study combines state of the art mass spectrometry techniques (i.e. shotgun proteomics and COFRADIC) and very recently described deep sequencing techniques (i.e. ribosome profiling : sequencing of ribosome captured mRNA) .

Abstract:
Tandem mass spectrometry (MS/MS) -based shotgun proteomics or specific COFRADIC (COmbined FRActional DIagonal Chromatography) technologies are very effective for large-scale proteomics or chromatographically isolated peptide class identification. Protein sequence database searching is the most commonly used technique for the identification of peptides following the acquisition of MS/MS spectra. Although convenient for routine use, these public databases are collections of all known and predicted proteins in a species and may not closely represent the real protein pool in a specific sample. In contrast, mRNA-seq derived translation products give a more representative expression state of the sample under investigation. Also, databases employed in proteomics searches are usually incomplete with respect to sequence variation information, such as single nucleotide variations (SNVs) and RNA-splice and -editing variants. Without taking them into account, proteomic studies may fail to detect novel, important protein forms. Surely, usage of mRNA-seq derived protein databases in the identification process has several advantages (as mentioned) and will become mainstream in due time.

Ribosome profiling (the deep sequencing of ribosome-protected mRNA fragments) is a new technique developed by Ingolia *et al.*, that allows precise and quantitative analysis of genome-wide *in vivo* translation. Ribosome profiling measures expression at the level of translation, opening new windows on *in vivo* translation. Recently, Ingolia *et al.* also described a strategy exploiting the propensity of harringtonine to cause ribosomes to accumulate at sites of translation initiation. Combination of the ribosome profiling mRNA-seq data with a machine-learning algorithm can define protein products systematically. On the other hand, Guttman *et al.* state that ribosome profiling has indeed provided us with a strategy for identifying ribosome occupancy on RNA, serving as a method to distinguish between coding and non-coding transcripts. However, this first needs to be tested because non-coding transcripts that show an association with the ribosome have not been shown to have a protein product.

To verify the potential of ribosome profiling in providing us with a true snapshot of the translational landscape, we generated a database of translation products based on the ribosome profiling experiments from the Ingolia *et al.* study on mouse embryonic stem cells. Also, we *in silico* derived a human version of this data. Several shotgun-proteomics experiments and N-terminal COFRADIC (isolating N-terminal peptides in the chromatography step) MS/MS result sets were searches against these compiled translation product databases. Extra MS experiments, trying to isolate small endogenous peptides in mouse embryo samples are still ongoing.

We were able to validate several findings of the initial ribosome profiling data. Several alternative translation start sites were identified, of which many with near-cognate start codon. We could also reaffirm N-terminal truncation and elongation products. Furthermore, ongoing experiments could possibly reveal true uORF (upstream open reading frame) and/or sORF (small ORF) translation products.

As deep sequencing techniques are becoming more standard, inexpensive, and widespread, the ribosome profiling methodology will in the future probably serve as an alternative to MS-based protein identification, moreover since its large dynamic range, sensitivity, and comprehensive nature. On the other hand, enrichment techniques preceding MS experiments (such as for example N-terminal COFRADIC) will still be indispensible to lower the complexity of the peptide mixture and enrich for selected peptide classes. Furthermore, MS-techniques in general, provide us with true evidence of the *in vivo* proteins/peptides and not to forget their post-translational modification status. Anyhow, combination of aforementioned deep sequencing and mass spectrometry techniques will definitely become mainstream in the future in revealing translation levels.

The first two authors contributed equally to this work.

Oral Session

Monday, 17th September

Main Hall

15:00 – 17:00

Session 6: Novel Approaches in Proteomics Analysis

Chair: Roman A Zubarev (Karolinska Institutet, Sweden)

S06-1620 Top-down mass spectrometry for comprehensive quantitative proteomics

16:20 – 16:40

Ljiljana Pasa-Tolic, Shawna M Hengel, Si Wu, Zhixin Tian, David Stenoien, Rui Zhao, Nikola Tolic, Joshua T Aldrich, Da Meng, Ronald J Moore, Errol W Robbinson

Pacific Northwest National Laboratory

Keywords:
top-down quantitative proteomics PTMs

Novel aspects:
High throughput quantitative top-down LCMS for global and targeted analyses

Abstract:
Combinatorial post-translational modifications (PTMs) , signal peptide cleavages, proteolytic processing and site mutations are all important biological processes that largely go undetected in traditional bottom-up proteomic analyses. While several PTMs are successfully identified using bottom-up methods, information including stoichiometry of modifications on a single protein, or presence of a combination of multiple modifications on a single protein isoform are impossible to infer from peptide-level data. Because most proteins in a typical global proteomic study are not identified with 100 % sequence coverage, it is not known whether the lacking sequence coverage is due to biologically relevant proteolytic processing events, sample preparation, or MS duty cycle. The potential information gleaned from top-down proteomic studies, or through integration of top-down and bottom-up approaches, is vast and, combined with recent improvements in MS instrumentation, is rapidly becoming an important avenue for proteomic studies. The work presented here will discuss specific examples demonstrating why top-down analysis is preferred over traditional bottom-up studies.

Typically PTMs are not identified in global bottom-up quantitative studies, despite the fact that a large number of proteins are known to contain biologically relevant PTMs. While top-down analyses provide this information, throughput and sensitivity have been limiting factors due to extensive pre-fractionation efforts required for adequate proteome coverage. To tackle these challenges, we have optimized commercially available, LCMS platforms for high-throughput, comprehensive and sensitive top-down quantitative analysis using an *E. coli* lysate. Of the 502 proteins confidently identified from a combination of ETD and HCD MS/MS experiments searched, over half contained PTMs. This approach has been successfully applied to other systems, including thermobacteria, cynobacteria, and *Salmonella*.

However, complications at both the experimental and data-analyses levels remain, particularly in the case of a large number of co-occurring PTMs and other modifications, demonstrating a need for further improvement in the top-down proteomics field. Histones contain a large number of combinatorial modifications, providing a significant analytical challenge. While many modifications are localized to the N-terminus, modifications span across the entire sequence emphasizing the need for top-down studies to accurately reflect the number of relevant isoforms. To this end, we have developed a histone specific two dimensional LC-MS/MS platform that enabled identification of over 700 histone isoforms from 7.5 µg of starting material in a single 24-hour analysis.

Bottom-up quantitative studies can provide information on several thousand proteins, however, depending on activated biological processes, data can be difficult to interpret when going from a peptide to protein abundance. For example, iTRAQ analysis of a human skin cell model was used to identify abundance changes and translocation in response to exposure to ionizing radiation. The global study identified several hundred proteins changing with statistical significance. However, proteins that underwent processing or modification as a result of radiation exposure required a more complex analysis. Filaggrin consists of several domains that are cleaved and translocate in response to specific stimuli. Because filaggrin subunits could be considered unique species, we broke down the observed peptides into the N-terminal nuclear targeting domain, internal domains or C-terminal domains. Quantitative analysis indicated that the N-terminal domain localized to the nucleus as expected, and decreased in abundance following radiation exposure. Additionally, peptide rollup to proteins that underwent extensive radiation dependent phosphorylation were analyzed separately. Differences in fold-change were observed when the peptides containing known phosphorylation sites were included/excluded. These extra steps required previous knowledge of biology involved, and would not have been required if a top-down analysis was employed. Examples featured here highlight the complexity of comparing peptide abundance values in the context of protein abundance, and suggest that future top-down studies may be required for comprehensive analysis of biological processes.

Oral Session

Monday, 17th September

Main Hall

15:00 – 17:00

Session 6: Novel Approaches in Proteomics Analysis

Chair: Roman A Zubarev (Karolinska Institutet, Sweden)

S06-1640

16:40 – 17:00

In silico Compensation of Instrumental Response Fluctuations Improves Label-free Quantification of High-Resolution Proteomics Data

Yaroslav Lyutvinskiy[1], Hongqian Yang[1], Dorothea Rutishauser[1], Hilkka Soininen[2], Roman Zubarev[1]

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Keywords:
label-free software accuracy Alzheimer

Novel aspects:
In silico method of instrumental response compensation improves accuracy of label-free quantification by post-acquisition data processing. The generality of the technique promises its widespread application in mass-spectrometry based quantitative methods.

Abstract:
Introduction:
In the analysis of human biopsies, particular bodily liquids, the biological variations of interest are often small (~10%), which poses high requirements on the accuracy of proteomics analysis, especially when it is performed by the label-free method. One of the main contributors to the inaccuracy of label-free LC/MS experiment is the variability in the instrumental response, which includes fluctuations in the electrospray current, instrument transmission and detection sensitivity. We developed *in silico* method of reducing this variation, and dramatically improve the precision of label-free proteomics analysis by post-processing.
For abundant proteins, precision of ca. 1 % (CV) is achieved in relative quantification of blood plasma proteins of ageing population, which allowed clear gender differentiation of pooled samples and 86% accurate differentiation of individual samples.

Method:
The compensation method is based on the observation that in many proteomics experiments, most of the proteome stays unchanged and only a relatively small number of proteins (10-30 %) change their abundance as a biological response to the stimulus.
Since the fluctuations of instrumental response affect all simultaneously eluting peptides to the same degree, peptides from the "unchanged" part of the proteome can be used as references for abundance alignment of other peptides eluting in a different LC/MS run within the same narrow time window (1 min). By statistical analysis of the multitude of simultaneously eluting peptide species in both runs, for any given retention time the average ratio is calculated between the two runs for the "unchanged" peptides. Thus ratio is used as a correction factor that compensates such time-varying artifacts as ESI current fluctuations.

Implementation:
The above compensation method was implemented in Visual C# 2010 as a part of Quanti, a label-free quantification workflow. First, MS/MS data from the whole experiment (2-200 individual Orbitrap LC/MS .raw files) are merged together with elimination of duplicates, and searched by Mascot. Then Quanti aligns retention times of peptides by minimizing the changes in the elution order in different runs. In each LC-MS run, chromatographic peak of every Mascot-identified peptide is integrated after performing charge and isotope deconvolution. Relative abundances of peptides for each pair of LC-MS run are corrected as described above. Final relative abundances are inferred by statistical analysis of the matrix of pairwise comparisons for all possible pairs of LC-MS runs.

Preliminary data:
Label-free quantification was applied to proteomic blood plasma analysis of 8 samples pooled from 218 age-matched persons according to gender and the stage of Alzheimer's disease (AD) (Control; Mild Cognitive Impairment, MCI; Progressive MCI; AD). Protein extraction and digestion was repeated three times for each sample. Each prepared peptide mixture was analyzed twice on Orbitrap Velos with Easy-nLC (both Thermo) using 90 min LC gradient. Altogether, 1441 peptides were identified with 1 % FDR. Label-free quantification was performed by Quanti. When technical replicates were compared, the average R^2 factor between peptide abundances was 0.98 for the linear abundance scale and 0.96 for the $\log_{10}$ scale. Application of instrumental correction improved the R^2 factor to 0.995 and 0.98 for the linear and logarithmic scales, respectively. For abundant proteins, precision of ca. 1 % (CV) is achieved.

Significantly, measured protein abundances after correction correlated better with known biological aspects of the samples. 100% accurate sex differentiation is achieved on pooled blood plasma samples. When the same proteins as in the pooled samples were used for sex differentiation in individual samples, correct sex identification from a single LC/MS analysis of each sample was achieved in 31 out of 35 cases (88% accuracy) , as opposed to 28 cases out of 35 (80% accuracy) without the correction.

Oral Session

Monday, 17th September

Room A

15:00 – 17:00

Session 7: New Ionization Methods and Related Topics for the Next Generation

Chair: Kenzo Hiraoka (University of Yamanashi, Japan)

S07-1500

15:00 – 15:40

[Keynote Lecture] What is the Opposite of Pandora's Box? Direct Analysis, Ambient Ionization, and a New Generation of Atmospheric Pressure Ion Sources.

Robert B Cody

JEOL USA, Inc.

Keywords:
DART, ambient, ionization, direct

Novel aspects:
Ambient ionization has led to development of many new ion sources. Direct analysis relies on the mass spectrometer selectivity, but chemistry and complementary methods can provide major benefits.

Abstract:
Ion sources have changed dramatically over the decades since chemical ionization was first introduced as an alternative to electron ionization. Game-changing developments such as FAB and plasma desorption were followed by the development of atmospheric pressure ion sources. Two revolutionary new ion sources became widely available in the early 1990's : ESI and MALDI suddenly made the analysis of large biomolecules possible for the first time.

The first ambient ion sources, DART and DESI, were first publicly presented in adjacent talks at the ASMS Sanibel Conference in January 2005. Both were new atmospheric pressure ion sources .The surprising aspect of what is now termed "ambient ionization " was that it was much easier to generate ions than we thought! Mass spectrometry could now analyze samples ionized in open air in the laboratory environment, with little or no sample preparation. This led to the development of a new generation of atmospheric pressure ion sources based on plasma techniques like DART, spray techniques like DESI, or hybrid methods combining multiple ionization mechanisms.

Our work with DART has led us to press the limits of direct ionization to see how far we can go in analyzing a wide range of simple and complex samples with minimal sample preparation. In some cases, the mass spectrometer provides enough information through high resolution and/or MS/MS to identify complete unknowns, even in complex mixtures. "Fingerprinting " of complex mixtures by DART has been used to identify biological species and to characterize commercial products.

However, no single method is ever sufficient to solve all analytical problems by itself. One must appreciate the limitations of a technique as well as its advantages and try to decide where improvements can be made. Taking advantage of simple chemical "tricks " such as in-situ derivatization, rapid sample cleanup methods, and in-situ H/D exchange, we have been able to ionize, and obtain more information for, a larger set of compounds than by DART alone. A better understanding of the ionization mechanism has allowed us to control the selectivity of DART for some analyses. After trying to find out how far we can go to analyze complex samples without chromatography, we found that reintroducing separations in combination with ambient ionization can provide some unique benefits.

A recent example has been the use of DART to identify new designer drugs that are suddenly appearing on the market in attempts to circumvent the laws governing drugs of abuse. In some cases, we have been able to identify specific designer drugs in so-called "herbal incense " or "herbal smoking mixtures " by DART alone. Exact mass measurements and isotopic abundances in combination with fragmentation may suffice for many known target compounds. However, complementary techniques including HPLC and NMR are needed to separate isomers and conclusively determine structure for new drugs for which standards are not available.

Oral Session

Monday, 17th September

Room A

15:00 – 17:00

Session 7: New Ionization Methods and Related Topics for the Next Generation

Chair: Kenzo Hiraoka (University of Yamanashi, Japan)

S07-1540

15:40 – 16:00

Temperature Dependence of Proton Transfer for Biomolecular Ions with Electrospray Ionization

Shinji Nonose, Kazuki Yamashita, Ayako Sudo, Keishi Machida, Kanako Yokoyama, Minami Kawashima, Takashige Mori, Yuto Ohshima

Yokohama City University, Yokohama, Japan

Keywords:
temperature dependence, proton transfer

Novel aspects:
Temperature dependence of proton transfer for isolated multiply-charged protein and peptide ions with gaseous molecules was investigated. Dramatic change was observed for distribution of product ions and absolute reaction rate.

Abstract:
The structures and reactions of gas-phase biological molecules bridge the gap between the gas phase and the real living system. In order to investigate the intra- and intermolecular interactions, nonvolatile molecules should be introduced into the gas phase as ions. These studies are expected to provide a deeper understanding of the intra- and intermolecular interactions that determine the conformations. In the present work, temperature dependence ofproton transfer for isolated multiply-charged protein and peptide ions with gaseous molecules of large proton affinity (PA) was investigated with mass spectrometry, which elucidates the synchronized processes in biomolecular systems.

A home-made tandem mass spectrometer with electrospray ionization (ESI) was used for measurements. Multiply-charged protein and peptide ions were produced by ESI of a dilute solution of proteins in methanol-water mixture including acetic acid. We choose lysozyme, cytochrome c, myoglobin, ubiquitin, insulin, hemoglobin and albumin as sample proteins, and choose angiotensin I, angiotensin II, bradykinin, substance P, somatostatin, neurotensin, melittin, insulin chain B, ACTH 1 -24, RKRARKE, KKK, KKKK and KKKKK as sample peptides. The ions produced by ESI are admitted into the vacuum chamber through stainless capillary. The charge-selected protein and peptide ions emerging from a quadrupole mass spectrometer are admitted into a collision cell with octapole ion trap. The collision cell is filled with He including gaseous molecules of large PA. We choose 1 -propylamine, 1 -butylamine, 1 -pentylamine, pyridine, tert-butylamine, diethylamine, dipropylamine, 2,6-dimethylpyridine, or triethylamine as target molecules. Temperature dependence of reaction rate and branching fractions for proton transfer from multiply-charged protein ions to the target molecules was measured, by changing temperature of collision cell. The parent and product ions are mass-analyzed by a time-of-flight mass spectrometer equipped with reflectron.

Proton transfer from the protein and peptide ions to the target molecules was occurred by collisions in the cell. Absolute reaction rate for proton transfer was estimated with intensity of ions in the mass spectra. In any proteins, the reaction rate increased rapidly with increasing number of the charges. By changing temperature of collision cell in region from 280 to 470 K, temperature dependence of reaction rate and branching fractions for proton transfer from multiply-charged protein and peptide ions to target molecules was measured. Dramatic change was observed for distribution of product ions and reaction rate. These results would correlate with conformation change of protein and peptide ions with change of temperature, which originates in self-solvation of the proton by hydrophilic residues in polypeptide chains, delocalization of charges with self-solvation, and Coulomb interaction between charges.

Oral Session

Monday, 17th September

Room A

15:00 – 17:00

Session 7: New Ionization Methods and Related Topics for the Next Generation

Chair: Kenzo Hiraoka (University of Yamanashi, Japan)

S07-1600

16:00 – 16:20

Design and Development of A Novel Nuclear Magnetic Resonance Detection for the Mass-selected Gas-Phase Ions by "Magnetic Resonance Acceleration" Technique

Kiyokazu Fuke[1,2], Masahide Tona[1], Akimasa Fujihara[2], Haruki Ishikawa[3]

[1]Kobe University, Kobe, Japan, [2]Osaka Prefecture University, Osaka, Japan, [3]Kitasato University, Kanagawa, Japan

Keywords:
NMR, mass-selected ion, ICR

Novel aspects:
This work is the first arttempt to detect nuclear magnetic resonace for mass-selected gas-phase molecular ions with a mass spectroscopic sensitivity.

Abstract:
Nuclear Magnetic Resonance (NMR) technique is a powerful tool to study the physical and chemical properties of materials in wide area. However, this technique is limited for the materials in condensed phase. Although NMR is also highly expected to use for mass-selected gas-phase ions in both fundamental and applied sciences, the method to extend to the gas-phase ions is not reported yet. In the present paper, we report a principle of the detection of NMR for the gas phase ions in a gradient magnetic field based on a "magnetic resonance acceleration " technique and describe the design and the construction of an apparatus, which we are developing. This technique is a new extension of a molecular-beam magnetic resonance method and is readily coupled with an ion cyclotron resonance mass spectroscopy (ICR) . Thus, in near furture, it may allow us to use for chemical analysis of mass-selected ions such as an ICR-NMR. We also present the experimental techniques and the results on the formation and manipulation of ultra cold ion packets in a strong magnetic field, which are the key techniques to detect NMR signal using the present method.

S07-1620

16:20 – 16:40

New prospects for post-ionization mass spectrometry using ultrafast laser pulses

Alessia Longobardo[2], Alisdair Macpherson[2], John Vickerman[1], Nick Lockyer[1,2]

[1]Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, UK, [2]Photon Science Institute, University of Manchester, Manchester, UK

Keywords:
Sputtered neutral mass spectrometry, photofragmentation, photoionization.

Novel aspects:
There are very few studies of molecular photoionization using femtosecond lasers in the IR wavelength range. Systematic study of this type is required to optimise post-ionization techniques.

Abstract:
Mass spectrometric techniques including secondary ion mass spectrometry and matrix assisted laser desorption ionisation have been very successfully applied in applications involving surface chemical analysis and imaging. Powerful though these approaches are, they rely on the detection of analytes ionized as a direct consequence of the desorption process. However, with very few exceptions, the dominant proportion of desorbed analyte species is neutral. The issue of enhanced ionization efficiency remains central to further developments in performance.

With regard to secondary ion mass spectrometry (SIMS), the introduction over the last decade polyatomic and cluster ion sources have improved the molecular sensitivity significantly, opening up new areas of application, particularly in the biosciences. However, the fraction of ejected species that are ionized in the SIMS process remains very small (typically 10^{-7}-10^{-3}) and is subject to large matrix ionization effects which limit routine quantification. Detecting the secondary neutral flux by post-ionizing analytes subsequent to desorption therefore offers potentially further increases in sensitivity and in ease of quantification. This is particularly important in imaging applications.

Laser post-ionization of sputtered neutrals has been very successfully applied for elemental analytes. For molecules the situation is complicated by photofragmentation and a detailed understanding of the ionization-dissociation characteristics of analytes is required to optimize the experimental parameters [1]. Recent work has shown that using femtosecond lasers with mid-IR wavelengths and/or ultrahigh intensity can overcome the traditional trade-off between molecular ionization efficiency and fragmentation [2].

Here we report on the efficiency of molecular ion and fragment ion production of a range of molecules as a function of laser wavelength and intensity. A clear transition in behaviour is observed favouring molecular ion production. This will be discussed in the context of the underlying mechanisms.

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S07-1640

16:40 – 17:00

Desorption/ionization induced by neutral clusters as matrix-free, soft, and efficient ion source for ion-trap mass spectrometry of biomolecules

Markus Baur[1], Bong-Jun Lee[1], Christoph R Gebhardt[2], Michael Durr[1]

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Keywords:
cluster, desorption/ionization, matrix-free, oligopeptides, ionization efficiency

Novel aspects:
Desorption/ionization induced by neutral clusters is a soft and matrix-free ionization source with high absolute ionization efficiency. Combination with ion-trap MS allows for high sensitivity and batch-type analysis of biomolecules.

Abstract:
Desorption and ionization induced by neutral clusters (DINeC) can be employed as a very soft and matrix-free method for transferring surface-adsorbed biomolecules into the gas phase. Using neutral clusters with polar constituents such as SO_2 or H_2O, the method makes use of the dipole moment of the cluster molecules which allows both for solvation and charge transfer processes in the cluster. Furthermore, shattering of the cluster during its impact on the surface leads to a rapid redistribution of the system's energy and an efficient cooling of the desorbed molecules. Thus the impacting cluster provides not only the energy for the desorption process but also serves as a transient matrix. As a consequence, desorption and ionization of oligopeptides and smaller proteins proceeds without any fragmentation of the biomolecules at comparably low energies of the impacting clusters [1].

In combination with ion trap mass spectrometry, desorption and ionization induced by neutral cluster impact can be utilized for soft and matrix-free, batch-type analysis of biosamples. Especially when the cluster beam is produced by a pulsed nozzle with pulse duration in the sub-millisecond regime, all ions generated during one pulse can be collected in the ion trap. In combination with an absolute ionization efficiency of the process on the percent level, this leads to a very low consumption of analyte material. E.g., for oligopeptides such as angiotensin II or bradykinin, the ionization efficiency was determined to be 3 to 4 % and femtomol sensitivity was achieved in the case of these oligopeptides. Furthermore, as no primary ions but neutral molecular clusters are used for the desorption and ionization process, the background intensity in the spectra is comparably low.

Due to the soft nature of the cluster-induced desorption process, the majority of the peaks in the initial spectrum can be directly associated with intact sample molecules ; in combination with full MS^n capabilities, structural analysis and straight-forward spectrum analysis is possible as illustrated for a set of rhodamine dyes.

The presented experimental setup is relatively simple and allows for easy combination of a DINeC source with standard mass spectrometers ; the process can be efficiently applied for a wide range of surface concentrations and configurations, i.e. from micrometer-thick films down to submonolayer surface concentration of biomolecules [2]. Samples are prepared by means of directly drop-casting the respective solution on a millimeter-size spot ; neither special sample preparation nor addition of matrix is needed. Multiple sample arrays and low sampling time then allows for batch-type analysis.

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Oral Session

Monday, 17th September — Room B-1

15:00 – 17:00

Session 8: Collision Dynamics and Spectroscopy Using Ion Storage Rings and Traps

Chair: Haruo Shiromaru (Tokyo Metropolitan University, Japan)

S08-1500 [Keynote Lecture] Spectroscopy of firefly luciferin and oxyluciferin anions in vacuo. Color tuning by a micro-environment?

15:00 – 15:40

Steen Brøndsted Nielsen
Aarhus University, Aarhus, Denmark

Keywords:
Ion spectroscopy, micro-environment, fireflies, luciferin

Novel aspects:
First gas-phase absorption spectra of luciferin and oxylucerin anions isolated in vacuo and the role of single water molecules

Abstract:
Luciferin is the substrate for the enzymatic reaction responsible for the characteristic yellow light emission from fireflies. In aqueous solution at physiological pH, the molecule is present in its anionic form. It undergoes an Mg^{2+}- mediated reaction with ATP in the active site of the luciferase enzyme. The resulting luciferyl adenylate compound reacts with O_2 to give the product anion, oxyluciferin, in a singlet excited state, which emits a photon of light as it relaxes to the ground state. Even though different species of bioluminescent beetles all use the same luciferin, the emission spectrum varies greatly, *e.g.*, the light emitted from the firefly *Photuris pennsylvanica* is green while that emitted by the railroad worm *Phrixotrix hirtus* is red. The most studied species, the North American firefly *P. pyralis*, produces a yellow-green light. The reason for this variability is up for much current debate. To better understand the bioluminescence phenomenon and environmental influences, my group has carried out gas-phase spectroscopy experiments on the bare luciferin and oxyluciferin anions to establish their electronic properties when isolated *in vacuo*. We have also studied the impact of single water molecules. Absorption spectra were obtained at the electrostatic ion storage ring in Aarhus and a sector instrument where ionic dissociation is monitored on a long time scale (up to milliseconds) and on a short time scale (few microseconds) , respectively. I will discuss the implications of our gas-phase results on the understanding of firefly bioluminescence. Finally, but not least, our data serve to benchmark theoretical models used to calculate excited states.

S08-1540

15:40 – 16:00

Electronic and photonic collision experiments on molecular ions with an electrostatic storage ring at KEK

Tetsumi Tanabe[1,3], Manabu Saito[2], Koji Noda[3], Evgeni B Starikov[4]

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Keywords:
storage ring, electron-ion collisions, biomolecule, photodissociation, interconversion

Novel aspects:
Electron-capture dissociation of S-S bonded peptide cations at high energies. Interconversion of tautomeric anions in vacuo.

Abstract:
The electrostatic storage ring at KEK is equipped with an electrospray ion source, an ion-trap, a merged electron beam device [1] and an OPO laser (wavelength : 410-600 nm) . The ring is also characterized by its ultra-high vacuum (10^{-11} Torr) , which realized long beam lifetimes resulting from less frequent collisions of stored ion beams with residual gas molecules. This makes it possible to perform electronic and photonic collision experiments on various molecular ions including biomolecules in an extreme environment. Here, we introduce some of the recent experimental studies using this ring.

It is well known that the electron-capture dissociation (ECD) is efficient for thermal electrons and the cross sections rapidly decrease with an increase of electron energies. Meanwhile, the ECD rate for peptide cations was also found to be significant for high energy (3-13 eV) electrons and this phenomenon is called 'hot electron capture dissociation' (HECD) [2] . Still, HECD reported so far are for peptides without disulfide (S-S) linkage [3] . Now we report on collisions of high-energy electrons with various protonated peptide monocations with disulfide bonds. Resonant neutral particle emissions at the energies of 6-7 eV were observed, as well as a rise towards zero-energy, which are typical electron-capture dissociation profiles. The presence of disulfide bonds tends to enhance the resonant bump heights. Chemical nature of the amino-acid residues adjacent to cysteines appears to correlate with the bump strength [4] .

Fluorescein and its derivatives are the most widely recognized dyes owing to their unique bright fluorescence. The monoanions are considered to adopt two basically possible tautomeric forms, i. e., the monoanionic carboxylate (MAC) and the monoanionic phenolate (MAF) forms. Whereas the photoabsorption properties of MAC have been extensively studied, there is a dearth of information regarding MAF. The photodissociation of fluorescein monoanions was studied by varying the storage times of the ions in the ion trap and the electrostatic storage ring, before laser irradiation. The photodissociation neutral spectra as a function of time consist of components with short and long lifetimes, and vary in a manner dependent on the storage time as well as the laser wavelength. By comparing the neutral yields as a function of wavelength obtained herein with the photoabsorption spectra of fluorescein reported recently for the solution and gas phases, it was deduced that the spectra originated from various tautomers of fluorescein monoanions. Moreover, interconversion of these tautomers occurs during long-term storage in the storage ring *in vacuo*. The wavelength spectra of the converted ions depend strongly on the storage time in the ring. In addition, we have also performed detailed semiempirical quantum-chemical computations, to try revealing what kind of monoanion tautomers we are dealing with [5] .

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Oral Session

Monday, 17th September — Room B-1

15:00 – 17:00

Session 8: Collision Dynamics and Spectroscopy Using Ion Storage Rings and Traps

Chair: Haruo Shiromaru (Tokyo Metropolitan University, Japan)

S08-1600
16:00 – 16:20

Cold chemical reactions between sympathetically cooled molecular ions and slow polar molecules

Kunihiro OKADA[1], Takahiro FURUKAWA[1], Masanari ICHIKAWA[1], Michiharu WADA[2], Hans SCHUESSLER[3]
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Keywords:
ion trap, ion Coulomb crystal, Stark velocity filter, ion-molecule reaction, polar molecules

Novel aspects:
We developed a novel method to directly measure cold ion-polar molecule reactions by using a Stark velocity filter and a sympathetic laser-cooling method, and applied to the measurement.

Abstract:
During the recent decades several techniques for producing cold atoms, molecules, and their ions have been developed, and they are now being applied to the study of cold atomic and molecular processes, such as cold ion-molecule reactions. In fact, a mearsurement of the reaction rate between a laser-cooled Ca^+ Coulomb crystal and slow CH_3CF molecules, produced by a Stark velocity filter [1], was demonstrated at about 1K [2]. The method can be extended to reaction rate measurements of cold molecular ion-polar molecule reactions, which play an important role in the chemical evolution of dark interstellar clouds [3]. Here we report on this extension and describe our apparatus and results to directly measure cold molecular ion-polar molecule reactions. The setup consists of a Stark velocity filter and a detection vacuum chamber enclosing a cryogenic linear rf ion trap. We have produced slow ND_3, CH_2O, and CH_3CN beams by using the Stark velocity filter. A time-of-flight method was applied to determine the velocity distribution of these slow polar beams. The typical peak velocity is 30 - 40m/s, and the number density of the polar molecules is determined to be $n = 10^4$ -10^6 cm^{-3} in the ion trapping region. Both these quantities are important parameters to determine the reaction rate constant of the ion-molecule reactions of interest. For preparing the cold molecular ions, sympathetic cooling by laser-cooled Ca^+ ions is applied in the cryogenic linear trap. In doing so we have successfully produced various Ca^+ Coulomb crystals including two-species Coulomb crystals, and in addition characterized them by molecular dynamics simulations [4, 5]. Recently, cold CaH^+, N_2^+ and N_2H^+ ions embedded in a Ca^+ Coulomb crystal have already been produced in our experiments. Before starting the reaction experiment with sympathetically cooled molecular ions, the reaction rate measurements between a Ca^+ Coulomb crystal and slow polar molecules were performed to check the reactivity of the slow polar molecules with the Ca^+ Coulomb crystal itself. As a result, the reaction rates are of the order of 10^{-6}- 10^{-5} s^{-1}, which are very slow compared to typical reaction rates of molecular ion-polar molecule reactions at very low temperatures. This fact shows that Ca^+ is well suited as a coolant when sympathetically cooling molecular ions, which are targeted for the reaction-rate measurements. Finally, we have performed initial reaction rate measurements between some of the above sympathetically cooled molecular ions and velocity-selected CH_3CN molecules. Preliminary results will be presented and discussed.

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Oral Session

Monday, 17th September — Room B-1

15:00 – 17:00

Session 8: Collision Dynamics and Spectroscopy Using Ion Storage Rings and Traps

Chair: Haruo Shiromaru (Tokyo Metropolitan University, Japan)

S08-1620 Development of a portable electrostatic ion storage ring in TMU

16:20 – 16:40

Jun Matsumoto, Haruo Shiromaru
Tokyo Metropolitan University, Tokyo, Japan

Keywords:
Electrostatic ion storage ring, large molecular ions

Novel aspects:
A portable electrostatic ion storage ring is being developed. It complements one-pass experiment apparatus and the conventional electrostatic ion storage rings in terms of an observation time range.

Abstract:
An electrostatic ion storage ring is a powerful device for investigation of slow reactions such as meta-stable decay and radiative cooling of large molecular ions including clusters and bio-molecules. We are developing a portable electrostatic ion storage ring (μE-ring) reducing ten times from the existing ring at Tokyo Metropolitan University (TMU), keeping the basic idea for beam manipulation unchanged. That is, the μE-ring consists of two 160 degree deflectors, four 10 degree deflectors and four focusing and defocusing electrostatic quadrupole doublets. It will store positive or negative ions with the energy range from several to 20 keV with the storage time up to several seconds under expected base pressure. Compact size of the ring has great advantages. A short period of revolution provides higher time resolution of observations to be made at the straight section of the ring. The portability enables us to bring it to various ion beam facilities. In addition, use of the ring in a synchrotron radiation facility will definitely expand the field explored by the ion-storage ring.

In the present study, first, we reconsidered transport property of an ion beam in order to check the effect of the miniaturization. Based on the equation of motion of ions in the ring, transfer matrices were produced, and the Twiss parameters were calculated. Then the stable conditions of betatron oscillations were derived by changing voltages of the quadrupole doublets. Second, actual electric fields generated by the electrodes were calculated by a simulation code for electric fields and the trajectories of charged particles, SIMION. The simulated ion beam trajectories show considerable deviation from ideal electric field. Therefore, the ion optics was refined with field clamps.

The dimensions of each electrode were determined with taking care of accuracies of machining and assemblage due to the miniaturization. In the final design, the circumference of an ion beam trajectory is about 0.8 m, and all electrodes are mounted on a single rectangle plate of 480 mm x 200 mm, for precise alignment of the electrodes and efficient bake-out of the ring. Now, the ring is under mounting operation. In the presentation, we will show the detailed design and the status of the μE-ring.

Oral Session

Monday, 17th September — Room B-1

15:00 – 17:00

Session 8: Collision Dynamics and Spectroscopy Using Ion Storage Rings and Traps

Chair: Haruo Shiromaru (Tokyo Metropolitan University, Japan)

S08-1640

16:40 – 17:00

Investigation of a novel apparatus which combines ion trap - laser cooling technique with ICP-MS for trace isotope analysis

Masanori Kitaoka, Yuta Yamamoto, Takuma Yoshida, Kyunghun Jung, Shuichi Hasegawa

The University of Tokyo, Tokyo, Japan

Keywords:
Isotope Analysis, Ion Trap, Laser Cooling, ICP-MS, Calcium

Novel aspects:
A novel apparatus which combines ion trap - laser cooling technique with ICP-MS is developed to be applied to trace isotope analysis.

Abstract:
Analysis of rare isotopes has applications in biomedicine, archeology and planetary science [1]. For instance, calcium has six stable isotopes, ^{40,42,43,44,46,48}Ca (abundance : 96.9%, 0.647%, 0.135%, 2.09%, 0.004%, 0.187%, respectively) and a long-lived radioactive ultra-trace isotope, ^{41}Ca (^{41}Ca/Ca = 10^{-15} - 10^{-14}). Today only AMS (Accelerator Mass Spectrometry) is utilized for practical purposes. In general, isobaric interference is an unavoidable issue for mass spectrometry. Therefore, collision/reaction cell, which is utilized to remove the isobaric ions by means of gas collisions or chemical reactions, has to be built in ICP-MS (Inductively Coupled Plasma Mass Spectrometry). However, the chemical reactions in a cell produce other isobaric ions. In the case of $^{41}Ca^+$, for instance, $^{40}CaH^+$ is produced when NH_3 is introduced to the cell for removing $^{40}Ar^+$ or $^{40}ArH^+$. Alternatively, laser-based methods, such as RIMS (Resonance Ionization Mass Spectrometry) and ATTA (Atom Trap Trace Analysis), have been developed for laboratory application [2]. We have developed a novel method which combines ion trap - laser cooling technique [3-5] with ICP-MS. Before using ^{41}Ca, we will demonstrate the principle of the method and evaluate the performance of our experimental system with the stable isotopes of Ca.

Our experimental system consists of ICP-MS, ion transport system, linear ion trap, laser system and observation system. Liquid sample is continuously injected to the ICP-MS and ionized by the Ar^+ plasma. Ions are transported through the collision/reaction cell and the quadruple mass separator. Mass-selected ions are extracted to the transport system, deflected 90 degrees and decelerated by collisions with buffer gas. Then the ions are loaded to a linear ion trap and laser-cooled. We employ External Cavity Diode Laser (ECDL) systems for generating 397 nm and 866 nm wavelengths to laser-cool Ca^+. To observe Ca^+, Laser Induced Fluorescence (LIF) is collected by an objective lens and projected to an Intensified Charge-Coupled Device (ICCD). Isotope selective observation can be achieved by means of the isotope shifts of the transition wavelengths.

We investigated the transport and trapping efficiency as functions of the flow rate of cell gas, the buffer gas pressure and the isotope species. We found that the cell gas worked not only for removing interference ions, but also improving the beam quality. Therefore, there was an optimum flow rate to maximize the number of ions extracted from ICP-MS. The dependence on the buffer gas pressure was investigated between 10^{-6} and 10^{-3} Torr. The loading and loss rate of trapped ions increased as the buffer gas pressure increased and 10^{-4} Torr was the optimum pressure to maximize the LIF. After the optimization of these parameters, all the stable isotopes of Ca including $^{46}Ca^+$, whose abundance is only 0.004 %, could be observed. To observe $^{43}Ca^+$, the hyperfine structure should be considered because of its nuclear spin. To create closed cycle, two 397 nm lasers were employed and a relatively high power 866 nm laser was utilized to excite the metastable levels. We will discuss the details of these properties of our new apparatus and the observation of all the stable isotopes of Ca.

References

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Oral Session

Monday, 17th September

Room D

15:00 – 17:00

Session 9: Imaging-II

Chair: Jiro Matsuo (Kyoto University, Japan)

S09-1500 [Keynote Lecture] Nanoscale Chemical Imaging of Biomaterials with Cluster SIMS

15:00 – 15:40

Nicholas Winograd
Penn State University

Keywords:
C60, Argon clusters, imaging, single cells, depth profiling

Novel aspects:
Imaging with mass spectrometry with 250 nm spacial resolution and 5 nm depth resolution. Metabolite distributions in single cells.

Abstract:
Bombardment of molecular solids with polyatomic projectiles allows interrogation of the sample with reduced chemical damage accumulation. Hence, it is now possible to perform depth profiling experiments with a depth resolution of less than 5 nm. In our hands, the projectiles of choice are C_{60} and argon gas cluster ions such as Ar_{2500}. To retain molecular information during beam-induced erosion, any damage accumulation must be removed at least as rapidly as it is formed. Here we discuss a number of fundamental descriptions associated with molecular depth profiling. These descriptions, which include both analytical models valuable in parameterizing the acquired signals and a molecular dynamics approach important for visualizing the action on a molecular level, point towards experimental conditions that optimize the quality of a depth profile. For example, the size and kinetic energy of the polyatomic projectile, the angle of incidence and the temperature all have significant influence on whether the important molecular ion signals are retained. Atomic force microscopy (AFM) is shown to be an essential technique for quantitative characterization of any molecular profile.

An important application of this mode of mass spectrometry is to provide chemical information for a wide variety of molecules in biological tissue and single cells. Although early attempts at implementing this application focused on low molecular weight fragment ions, polyatomic primary ion sources have opened detection schemes to a much wider range of molecules with molecular weights extending to greater than 1000 Da. In this regard, a number of workers have reported the distribution of drug molecules and of metabolites under various conditions. Here we discuss a number of challenges facing this field given the new measurement paradigms. Very different sample preparation measurement considerations emerge as the desired spatial resolution approaches 1 micron. At values above 1 micron, freeze drying methods appear to be successful and there are enough molecules in the probe area to detect higher mass species directly. For example, we have detected the molecular ion of more than 50 different lipids in brain tissue and in a lawn of cells, using ms/ms techniques to assign structure. Characterization of single cells becomes much more challenging, however, since there are many fewer molecules available for detection, and sample pretreatment to enhance ionization can move molecules around, reducing the effective lateral resolution. For these systems, preparation of frozen hydrated samples appears to be the best way to avoid artifacts. Moreover, since polyatomic projectiles now allow molecular depth profiling, use of frozen hydrated samples is the only way to preserve 3-dimensional structure of cells. Currently, using a conventional high performance imaging TOF-SIMS system, the sensitivity is on the limit of being able to detect the higher mass molecular ions. Several examples will be given to illustrate each of the above issues. The prospects for improving the sensitivity of this type of imaging will also be discussed.

Oral Session

Monday, 17th September

Room D

15:00 – 17:00

Session 9: Imaging-II

Chair: Jiro Matsuo (Kyoto University, Japan)

S09-1540

15:40 – 16:00

TOF-SIMS imaging analysis of atherosclerotic aortic sinuses and gastric cancer tissues

Eun-Soo Lee[1,2], Ji-Won Park[1,2], Se-Hwa Kim[1], Hyun Kyong Shon[1], Sohee Yoon[1], JuYeon Oh[1], Tae Geol Lee[1,2], Hark Kyun Kim[3], Daehee Hwang[4], DaeWon Moon[1,2]

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Keywords:
TOF-SIMS, Atherosclerosis, Gastric Cancer, Spectroscopic Mass Imaging, Ar gas cluster ion beam

Novel aspects:
1) . TOF-SIMS imaging applications to biomedical tissues such as atherosclerotic aortic sinuses and to gastric cancer tissues 2) Development of SIMS/MALDI microscopic mass imaging system for high throughput imaging.

Abstract:
Time-of-flight secondary ion mass spectrometry (TOF-SIMS) has been a useful tool to profile secondary ions from the near surface region of specimens with its high molecular specificity and submicrometer spatial resolution. We have been trying to develop TOF-SIMS into a molecular histology tool, which can be a practically useful biomedical diagnostic for clinical applications in cardiovascular and oncology areas. Summaries of TOF-SIMS applications to map the distribution of cholesterol ester in atherosclerotic aortic sinuses and to discriminate gastric cancer tissues from normal tissues with identification of biomarker candidates associated with the amino acid metabolism

Changes in cholesterol ester (CE) content regulate the progression of atherosclerosis. However, the spatial dynamics of CE subsets and their quantitative changes during lesion progression are not well understood due to a lack of appropriate imaging techniques. In this study, we developed an imaging based analysis method to map the distribution of CE subsets using ToF-SIMS. Serial sections of atherosclerotic aortic sinuses from apolipoprotein E knock-out mice (n=15) fed a 0.15 % high-fat diet for 1220 weeks were examined by ToF-SIMS. We found that the ratio of cholesteryl palmitate (Ch-PA) to cholesteryl oleate (Ch-OA) increased by approximately 99% (p=0.02) as atherosclerosis progressed, whereas the ratios of cholesteryl linoleate (p=0.09) and cholesteryl stearate (p=0.22) to Ch-OA did not change significantly. In advanced atherosclerotic plaques, *in situ* cell death assays showed that local Ch-PA densities were highly correlated with an increase in the number of apoptotic cells. These results suggest that increased Ch-PA may contribute to the formation of a necrotic core by increasing cell death. Our results indicate that the regional ratio of CEs as measured by ToF-SIMS might be a valuable new marker of atherosclerotic progression.

The TOF-SIMS analysis of even a moderately large size of samples has been hampered due to the lack of tools for automatically analyzing the huge amount of TOF-SIMS data. Here, we present a computational platform to automatically identify and align peaks, find discriminatory ions, build a classifier, and construct networks describing differential metabolic pathways. To demonstrate the utility of the platform, we analyzed 43 data sets generated from seven gastric cancer and eight normal tissues using TOF-SIMS. A total of 87 138 ions were detected from the 43 data sets by TOF-SIMS. We selected and then aligned 1286 ions. Among them, we found the 66 ions discriminating gastric cancer tissues from normal ones. Using these 66 ions, we then built a partial least square-discriminant analysis

(PLS-DA) model resulting in a misclassification error rate of 0.024. Finally, network analysis of the 66 ions showed disregulation of amino acid metabolism in the gastric cancer tissues. The results show that the proposed framework was effective in analyzing TOFSIMS

data from a moderately large size of samples, resulting in discrimination of gastric cancer tissues from normal tissues and identification of biomarker candidates associated with the amino acid metabolism.

Finally, TOF-SIMS for biomedical applications is still suffered from insufficient molecular ion yields, which may be overcome with an Ar gas clusters ion beam. For clinical applications, high throughput is critical to make the diagnosis technique inexpensive. KRISS has been tackling these two issues by developing a TOF-SIMS/MALDI microscopic mass analysis system based on an Ar gas cluster ion beam and a broad laser beam. Recent preliminary progresses will be reported and discussed together.

Oral Session

Monday, 17th September

Room D

15:00 – 17:00

Session 9: Imaging-II

Chair: Jiro Matsuo (Kyoto University, Japan)

S09-1600 Diagnostic application of Imaging Mass Spectrometry

16:00 – 16:20

Mitsutoshi Setou[1], Kiyoshi Ogawa[2], Akiko Kubo[3], Ikuko Yao[4], Masaaki Matsuura[5]

[1]Hamamatsu University School of Medicine, [2]Shimadzu, [3]Keio Universiy, [4]Kansai Medical University, [5]Japanese foundation for cancer research

Keywords:
imaging diagnosis biopsy pathology

Novel aspects:
meta analysis of histopathological lipidomics with high resolution imaging mass spectrometry

Abstract:
We developed high resolution imaging mass spectrometry system and determined quantitative and qualitative alterations in lipids. Molecular species of phosphatidylcholine (PC) on thin slices were determined during the occurrence and progression of diseases in patients and model animals to identify potential clinical indicators of pathology. In addition, species of phospholipids were quantified by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) of lipid extracts from control/patients. Unique distribution patterns were observed for phospholipids with different fatty acid compositions, and distinct dynamic changes were seen in both their amounts and their distributions in tissue as tissue damage resulting from diseases. The analysis was applied for various diseases such as colon cancer, gastric cancer, inflammatory bowel diseases, Alzheimer disease, schizophrenia, atherosclerosis, and so
on. The specificity and generality of the lipid changes in human pathology revealed by the meta-analysis will be presented and discussed.

Oral Session

Monday, 17th September

Room D

15:00 – 17:00

Session 9: Imaging-II

Chair: Jiro Matsuo (Kyoto University, Japan)

S09-1620

16:20 – 16:40

High-throughput analysis for metabolic dynamics and in situ metabolite imaging by MALDI mass spectrometry

Daisuke Miura[1], Yoshinori Fujimura[1], Shin-ichi Yamaguchi[2], Noriyuki Ojima[2], Mitsuru Shindo[1], Hiroyuki Wariishi[1]

[1]Kyushu University, [2]Shimdzu Co., Kyoto, Japan

Keywords:
MALDI-MS, High-throughput, Metabolite imaging, Synthesized matrix

Novel aspects:
Our developed MALDI-MS techniques combined with newly synthesized matrices may be useful for the advances in systems biology approach and biological or biomarker discovery.

Abstract:

Introduction

Mass spectrometry (MS) -based techniques such as LC-MS or GC-MS have been known to be a conventional strategy for low-molecular-weight metabolite analysis. However, these methods have drawbacks in analysis of tissue samples due to a requirement of metabolite extraction that causes loss of information on spatial localization. Moreover, total analysis time is still on the order of several minutes for a sample. Matrix-assisted laser desorption ionization (MALDI) has recently been reported to be applicable for low-molecular-weight metabolite analysis. Direct MALDI-MS analysis dose not require pre-separation step, and then the analysis time is within a minute for a sample. In addition, MALDI system enables us to analyze tissue sample directly (mass spectrometry imaging ; MSI) . It is generally known that the detectable molecules in MALDI-MS are completely dependent on the matrix used. Although there is a critical issue, limitation of the number of detectable analytes, in MALDI system, screening or development of novel matrix is believed to improve such drawback in coverage.

Herein, we report the development of MALDI-MS system towards the rapid analysis of metabolic dynamics and tissue metabolite imaging. Furthermore, we also describe an attempt to develop novel matrix for expanding the coverage of detectable metabolites in MALDI-MS.

Methods

For high-throughput analysis, *E. coli* was used as a model organism. Cell suspensions were directly mixed with matrix solution (5 mg 9-aminoacridine (9-AA) in methanol) for rapid sample preparation. This suspension were spotted onto MALDI sample plate and analyzed directly. For MSI experiment, rodent brain tissues were sectioned at 10 μm thickness with cryostat and then thaw-mounted onto an ITO-coated glass slide. A matrix solution was applied by spray-coating or sublimation method. In both types of experiments, data were acquired in negative ionization mode and signals between m/z = 50 to 1,000 were collected. Metabolites were identified or estimated by comparing MS/MS spectra with standard compounds or databases online database. For matrix development, more than 40 compounds including N- or C-substituted 9-AA analogues, anthracene derivatives and quinoline derivatives, were newly synthesized as matrix candidates. Using these synthetic matrices, 230 kinds of low-molecular-weight metabolite standards were measured by MALDI-MS in negative ionization mode.

Results and Discussion

Firstly, we demonstrated the feasibility of a high-throughput MALDI-MS system for tracing intracellular metabolic dynamics of bacteria. In a model system using *E. coli*, the time-dependent metabolite change was observed during environmental carbon source perturbation following the rapid relief from glucose limitation. This technique enabled to trace time-dependent transitions of the levels of phosphorylated metabolic intermediates and corresponding cofactors. Our proposed high-throughput analytical platform was expected to easily apply for a large-scale data acquisition of metabolite levels responsible for the central metabolism. Metabolite correlation network analysis using these data is now under way. Furthermore, this highly sensitive metabolite analysis technique was applied for in situ metabolite imaging. We could simultaneously visualize the distributions of a broad range of metabolites including nucleotides, cofactors, phosphorylated sugars, amino acids, lipids, and carboxylic acids in normal mouse brain tissue with single-cell sensitivity. Although we showed the findings using 9-AA as a matrix, recently several new synthetic matrices were found to be more efficient than 9-AA, depending on the metabolites. Detailed results will be discussed in the session.

(1) Miura D. *et al. Anal. Chem.* (2010) 82, 498-504. (2) Yukihira D. *et al. Anal. Chem.* (2010) 82, 4278-4282. (3) Miura D. *et al. Anal. Chem.* (2010) 82, 9789-9796. (4) Miura D. *et al. J. Proteomicsin press*

S09-1640 Imaging of metabolites in plants and insects with high resolution AP-MALDI MSI

16:40 – 17:00

Dhaka R Bhandari, Andreas Roempp, Bernhard Spengler

Justus Liebig University Giessen, Giessen, Germany

Keywords:
Imaging mass spectrometry, metabolites, high mass resolution and spatial resolution.

Novel aspects:
High resolution in space and mass method for understanding metabolites distribution in plants and insects.

Abstract:
Mass spectrometry imaging (MSI), introduced in the mid-90s, has shown encouraging progress recently in explaining the molecular basis of biological systems and processes. The technique has developed to become a promising tool for clinical investigation and biomolecular research (1-3). Herein, we present mass spectrometry images generated from plant and insect tissues that reveal spatial distributions of metabolites with high resolution in mass and space (HR^2). MSI is able to visualize the distribution of metabolites with high spatial resolution, aiding to explain mechanisms of complex biological processes.

A home-built atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization (SMALDI) ion source for imaging was used for ion generation (1). To acquire uniform sections from the sample, embedding materials such as carboxymethyl cellulose (CMC) and tragacanth gum were used for cryosectioning of insects. For root samples, on the other hand, sections were prepared manually using a common shaving blade. Pneumatically assisted spraying was employed for matrix deposition on the tissue, using a dedicated home-built apparatus. Matrices 2,5-dihydroxybenzoic acid (DHB) for positive ion mode and p-nitroaniline (pNA) for negative ion mode were used. The SMALDI source was operated with a nitrogen laser at 337 nm wavelength with a 60 Hz repetition rate. The source was coupled to an orbital trapping mass analyzer (Exactive or QExactive mass spectrometer, Thermo Fisher Scientific GmbH, Bremen) set to a mass resolving power of 50,000 to 140,000 at $m/z = 200$. The home-built software package 'Mirion' was used to generate mass images from the raw files generated by the mass spectrometer. Ions formed by 30 laser pulses were accumulated prior to detection, and matrix cluster peaks were used for internal calibration. All measurements including mass spectra from 10 micrometer pixels were acquired with a mass accuracy 2.5 ppm (root mean square). Molecular images were generated with a bin width of $m/z = 0.01$.

Images from seeds (rape and wheat), roots (rice and wheat) and insects (*Paederus* and *Harmonia*) were generated with 5 to 25 micrometer spatial resolution. For rapeseed at 10 micrometer pixel size, it was possible to image the ion at $m/z = 496.24$ (cyclic spermidine conjugate) that is only exhibited in the germinating region of the seed. For wheat seeds, several different metabolites were imaged which were located in different regions of the seed. Whole insect imaging of *Paederus* was performed at 10 micrometer spatial resolution and the defensive agent pederin, known to be produced by *Paederus*, was observed as sodium adduct at $m/z = 526.29$, as well as its metabolite pseudopederin with high mass accuracy. Similarly for *Harmonia*, MS images were generated using the signal of the resistance-related substance harmonine ($m/z = 283.31$), as well as using the signals of its metabolites, detected in different regions of the insect.

Literature：
1. Koestler et al. (2008) Rapid Commun Mass Spectrom. 22 (20)：3275-3285.
2. Roempp et al. (2010) Angewandte Chemie International Edition. 49 (22)：3834-3838.
3. Roempp et al. (2011) Anal Bioanal Chem. 401 (1)：65-73.

Oral Session

Monday, 17th September

Room E

15:00 – 17:00

Session 10: Ion Mobility Spectroscopy Based on Instrument & Theoretical Development

Chair: Toshiki Sugai (Toho University, Japan)

S10-1500 [Keynote Lecture] Pushing the frontier of high-definition ion mobility spectrometry using FAIMS

15:00 – 15:40

Alexandre A Shvartsburg, Gordon A Anderson, Richard D Smith

Pacific Northwest National Laboratory

Keywords:
IMS, FAIMS, isomer separation, PTMs

Novel aspects:
Novel waveform generators and gas compositions raise FAIMS resolving power by >10 times (to ~500 for peptides), enabling new approaches to proteomics (especially for characterization of post-translational modifications) and metabolomics.

Abstract:
Fast gas-phase separations using ion mobility spectrometry (IMS) prior to mass spectrometry (MS) are becoming ubiquitous, as the recent introduction of IMS/MS platforms by multiple major vendors rapidly expands applications into new areas. As single-stage drift-tube IMS analyses developed since 1970-s turn routine, research and technology development are moving into novel approaches beyond simple ion separation by low-field mobility.

The mobility of all ions in gases depends on the electric field intensity, and a major new IMS paradigm is differential or field asymmetric waveform IMS (FAIMS) based on the difference between mobilities at two intensities. That difference is often substantially uncorrelated to the ion mass/charge ratio or absolute mobility at either intensity, rendering the FAIMS dimension significantly orthogonal to MS or conventional IMS. While FAIMS/MS had been reported ~20 years ago and found promising for biological analyses, its utility was severely restricted by low resolving power, *R* (typically ~10).

We have strived to improve the *R* metric and resolution of FAIMS and explore the new application classes afforded by such gains. Modeling and experiments have revealed that high resolution requires homogeneous electric fields established in planar electrode gaps. New planar-FAIMS devices employing helium/nitrogen buffers with up to 75% He or power supplies that produce waveforms with amplitudes (dispersion voltage, DV) up to 5.4 kV have raised *R* for multiply-charged peptides to ~200, but electrical breakdown in gas precluded further increases of He fraction and/or DV. The resolving power of separations in media, including FAIMS, generally scales as the square root of separation time. Extending ion residence in the gap from the previously longest 0.2 s up to 0.8 s had raised the resolving power by up to twofold (to >300 for peptides). Hydrogen has much higher breakdown threshold than He, and the greatest H_2 fraction at maximum DV in our FAIMS unit is 90% vs. 50% for He. Hydrogen-rich mixtures allow improving the resolution further, especially for larger singly-charged ions, while accelerating analyses. Finally, new high-definition voltage generator with a more stable and precise output has narrowed the features at highest resolving power by ~1/3, pushing the maximum *R* to ~500.

Upon reviewing the latest advances in FAIMS technology, we shall discuss exemplary applications to proteomics and metabolomics. As a filtering technique, FAIMS is best suited for targeted analyses, such as separation and identification of isomeric peptides with differently localized post-translational modifications (PTMs). High-resolution FAIMS can broadly separate these variants for many modifications, including phosphorylation, glycosylation, and even smallest PTMs like acetylation or methylation, and various attachment sites. The capability extends to larger "middle-down" (~3 - 4 kDa) peptides such as histone tails that are richly modified and potentially exhibit millions of localization variants. Crucially, the variant separation is unrelated to the PTM shift involved and even those with adjacent alternatively modified sites (which challenge LC and MS/MS methods most) are well-resolved. Separations for different charge states are also independent, and the variants "co-eluting" for one are often resolved for another. The peak capacity is thus multiplied by the number of observed states, which is increasingly consequential for larger peptides that exhibit more states.

We shall also look at lipidomic applications, where FAIMS can assist lipid classification and effectively resolve regioisomers differing in the fatty acid position or other details.

Oral Session

Monday, 17th September — Room E

15:00 – 17:00

Session 10: Ion Mobility Spectroscopy Based on Instrument & Theoretical Development

Chair: Toshiki Sugai (Toho University, Japan)

S10-1540
15:40 – 16:00

Mobility of atomic and small molecular ions in cooled helium gas for investigation on interaction potentials and collision dynamics

Hajime Tanuma[1], Ryosuke Isawa[1], Jun-ichi Yamazoe[1], Shiro Matoba[2]

[1]Tokyo Metropolitan University, Tokyo, Japan, [2]Japan Atomic Energy Agency, Takasaki, Japan

Keywords:
ion mobility, interaction potential, collision dynamics

Novel aspects:
Extensive analysis of atomic and molecular ion mobiliy using ab initio potentials and two-temperature theory has revealed the collision dynamics at very low energy.

Abstract:
Typical measurements of ion mobility spectrometry (IMS) for chemical analysis have been performed in ambient air. Because the ambient atmospheric pressure is high of 0.1 MPa, the reduced electric field strength E/N in the drift tube is smaller than 1 Td even if the electric field is stronger than 100 V/cm. Therefore, the dependence of ion mobility on E/N is not important for IMS and the zero-field mobility has been used for the identification of the chemical compound. On the other hand, the traditional ion mobility measurements in rarefied gases have been performed in various electric fields and gas pressures, and the ion mobility as a function of the E/N has been investigated to discuss the interaction potentials and the elastic collision cross sections between the ion and the gas molecule.

In Tokyo Metropolitan University, we have developed a drift tube mass spectrometer operated at liquid helium and nitrogen temperatures, and measured the mobility of various atomic and small molecular ions at low temperatures. For the closed-shell systems, only one potential energy curve as a function of the internuclear distance is involved in elastic collisions. Therefore the measured ion mobility of the alkali ions, namely Li^+, Na^+, K^+, Rb^+ and Cs^+, in helium at 4.3 and 77 K can be reproduced by the classical calculation based on the two-temperature theory with the ab initio interaction potentials between the ions and helium.

For the open-shell systems, the interaction potential curve on the ground electronic state could split into different molecular states at the small internuclear distance. Using the averaged cross sections for two interaction curves, the mobility of the open-shell ions in helium can be calculated approximately. We have measured the mobility for C^+, N^+, O^+, He^+, Ne^+, Ar^+, Kr^+, and Xe^+ ions, and the calculation with the ab initio potentials shows fairly good agreement with the experimental results.

Not only atomic ions, diatomic and triatomic molecular ions have been used as the incident ions for the selected-ion-injection drift tube experiments. The molecular ion mobility as a function of the E/N shows quite different behavior from that of the atomic ions. For the atomic ions, the theoretical calculation of the mobility can be performed shortly. On the other hand, the mobility of molecular ions is extremely difficult to be calculated because of the multi-dimensional potential surfaces. To analyze the molecular mobility, the classical trajectory calculations have been carried out for N_2^+ and O_2^+ ions in helium, and the experimental results of the mobility can be reproduced quite well. In these calculations, it was found that the temporal rotational excitation plays a very important role.

Recently, we have measured the mobility of OH^+ and OD^+ in helium and observed a minimum in the mobility as a function of the E/N. The features of these results are quite different from those of O_2^+, CO^+, NO^+, CO_2^+, and NO_2^+, which have no minimum. The possible mechanism in collisions at few meV will be discussed with the ab initio potentials of molecular ions between helium atom.

We have also make a start on the development of an apparatus for the IMS in ambient air. The progress report on this new project will be given in our presentation.

Oral Session

Monday, 17th September — Room E

15:00 – 17:00

Session 10: Ion Mobility Spectroscopy Based on Instrument & Theoretical Development

Chair: Toshiki Sugai (Toho University, Japan)

S10-1600 Novel analytical approaches enabled by ultra-fast chip-based FAIMS separation coupled to mass spectrometry

16:00 – 16:20

Michael Ugarov[1], Yuqin Dai[1], Michael Flanagan[1], Michelle Romm[2], Vaughn Miller[2]

[1]Agilent Technologies, Santa Clara, USA, [2]Agilent Technologies, Wakefield, USA

Keywords:
FAIMS, ion mobility spectrometry, high speed separation, microscale fabrication

Novel aspects:
Novel approaches to applications enabled by high speed FAIMS separation

Abstract:
There has been a considerable increase in interest for orthogonal gas phase separations in combination with mass spectrometry during the last several years. High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) uses variation of ion drift velocity in atmospheric pressure environments at very high electric fields. It is often seen as a candidate for enhancing the separation provided by liquid chromatography (LC), or even potentially replacing LC stage entirely for some applications.

Previously, one disadvantage of all differential mobility devices has been the scanning duty cycle relative to fast LC. It proved a challenge to achieve performance that allows simultaneous effective separation, high transmission and scan speed. For this presentation we have characterized performance of a new generation of microscale FAIMS devices, which enable this ability and which promise to revolutionize high throughput multi-dimensional separations. Several mass spectrometry applications are expected to benefit from high speed pre-separation. We describe preliminary experimental results related to some of these potential applications.

A newly designed chip-based FAIMS device from Owlstone, Ltd., UK has been incorporated into the Agilent TOF mass spectrometry system. This generation of chip FAIMS devices has an analytical gap of 100 μm and ion drift path of 700 μm. The device comprises multiple channels with parallel electrodes designed to achieve optimum ion collection and transmission. In addition, this configuration offers enhanced selectivity while maintaining exceptional speed of separation. Entire CV scan can be accomplished in under 100 μs, while switching delay between different CV settings can be less than 5 μs. This speed is enabled by using very high values of dispersion field inside miniature channels.

As a result, the new device is compatible with TOF MS and QQQ MS data acquisition rates, as well as time scales of ultra-fast chromatography and solid phase extraction (SPE) based analytical methods such as Agilent RapidFire.

Preliminary testing has demonstrated that the performance is in good agreement with theoretical expectations. We report significant improvements in separation power and transmission, particularly for the high mobility ions compared to the previous series of prototypes. In addition, the FAIMS device demonstrates very low ion losses in "off-line" mode which supports high performance of the mass spectrometer in its regular operation regime. High dispersion voltages and short ion residence times are shown to provide high stability and reproducibility of operation with respect to specific conditions of analytical sample, solvent or chemical matrix.

Several potential applications have been demonstrated using the unique fast scanning and switching capabilities of the new system. They include separation of drugs, metabolites and interfering compounds, isomer identification, as well as peptide selection based on charge state distribution.

For example, the ability to separate components of a peptide mixture by charge state allows enhanced detection of higher charge ions with respect to singly charged ions as well as chemical noise. This proves beneficial for the identification confidence. This application requires adjustable resolution, excellent transmission and the possibility to switch dispersion and compensation field settings quickly to fully realize this potential benefit.

In another example, we were able to demonstrate conditions for selective transmission of phenacetin vs. acetaminophen, morphine vs. morphine glucuronide as well as some other pairs of analytes. This capability is very attractive, for example, in high throughput SPE/MS analytical methods such as RapidFire/MS which do not include liquid chromatography. Incorporating the FAIMS device into the RapidFire QQQ MS system provides an orthogonal mode of separation allowing more accurate quantitation of analytes that are susceptible to interference from in-source fragmentation.

Oral Session

Monday, 17th September — Room E

15:00 – 17:00

Session 10: Ion Mobility Spectroscopy Based on Instrument & Theoretical Development

Chair: Toshiki Sugai (Toho University, Japan)

S10-1620
16:20 – 16:40

Real-Time 2D separation with LC-DMS-TOF-MS: Increasing the Selectivity of High Resolution MS in Qualitative and Quantitative Analysis

J C Yves Le Blanc, Larry Campbell, Nic Bloomfield, Alexander V Loboda, Brad Schneider, Igor V Chernushevich

AB SCIEX, Concord, Canada

Keywords:
Quadrupole-TOF MS ; Differential mobility spectrometry ; LC-MS ; Selectivity

Novel aspects:
Improving high resolution MS detection of isobaric species based on atmospheric pressure differential mobility and liquid modifiers

Abstract:

Introduction

Complex samples mixture of low molecular weight and polar compounds are particularly challenging from an LC-MS acquisition perspective. In order to extract all relevant analyte information from such samples, higher peak capacity is frequently required, and this leads to coupling of comprehensive two-dimensional (2D) separation technique such as GCxGC and LCxLC. However, in such coupling, the second dimension of separation requires to be very fast, which can be challenging to LC. Here we propose that the second dimension can be obtained with chemically-assisted differential mobility separation (DMS) to provide more comprehensive detection of analyte from complex mixture. DMS provides a separation that is orthogonal to MS as well as LC, and the addition of chemical modifier in the transport gas can increase significantly the separation power of the DMS system.

Methods

A DMS cell was mounted in the atmospheric region between the entrance aperture of a hybrid quadrupole-time-of-flight mass spectrometer and TurboV® source. Nitrogen was used as the transporter gas, and constant gas flow in the DMS cell was achieved by the primary stage vacuum pumping of the MS system. Addition of 1.5% of either isopropanol (IPA) or acetonitrile (ACN) to the curtain gas was used to alter the DMS separation. LC was performed using a Shimadzu UFLC system operated at 400uL/min with a 2 x100mm (2.6u) Luna Kinetex C18 column. A mixture of 30 compounds ranging from m/z 70 to 700, with different polarity,was spiked in different samples matrix such as urine and protein precipitated plasma.

Preliminary Data

The DMS cell was operated at a separation voltage (SV) up to 4000 and the compensation voltage (CoV) was stepped from -30 to +10V with increments of 0.5V (81 steps). Mass spectra were collected at each step for 20ms, thus producing a total cycle time of 1.62sec. Using a gradient of 1 min from 2 % to 98 % organic, several of the lower mass compounds co-eluted. For species of lower mass ($m/z < 350$), a wide spread of CoV values were observed and many of the isobaric and co-eluting species were effectively separated as a result of using the DMS with either IPA or ACN as modifier in the gas transport. It is noticeable that some of the species that could not be separated by LC, such as fendiline (m/z=316.2060) and chlorprotixen (m/z=316.0912) were separated by the DMS, which enabled individual MS/MS data collection with precursor ion selection window equivalent to <0.1amu, as opposed to generating composite MS/MS spectrum. When dealing with samples of higher complexity, such as diluted urine samples, the DMS with IPA provided the highest peak capacity that complemented LC selectivity. This enables faster and more comprehensive coverage of analyte ions detected from complex samples while maintaining rapid sample throughput (i.e. rapid LC gradient). Additional results pertaining to selectivity gains for qualitative and quantitative analysis of TOF-MS and TOF-MSMS data will be presented.

S10-1640

16:40 – 17:00

Isomer-selected photodissociation of carbon and silicon cluster ions with ion mobility mass spectrometry using a double reflectron time-of-flight mass spectrometer

Kiichirou Koyasu, Ryoichi Moriyama, Tomohiro Ohtaki, Jun Hosoya, Fuminori Misaizu

Tohoku University, Sendai, Japan

Keywords:

Ion mobility spectrometry ; Carbon cluster ; Silicion cluster ; Photodissociation ; Double reflectron mass spectrometer

Novel aspects:

We have developed a combined setup of an ion drift cell with a double reflectron time-of-flight mass spectrometer, for the study of highly-sensitive isomer-resolved photodissociation with the tandem reflectrons.

Abstract:

In general, the number of coexisting isomers in atomic and molecular clusters increases rapidly with their sizes. Among such clusters, silicon cluster cations, Si_n^+, have stable prolate structures for $n< 30$ and spherical isomers for $n> 24$. Carbon cluster cations, C_m^+, also have different type isomers depending on their size ; cyclic isomers for $m< 40$ and fullerene isomers for the even number size at $m> 30$. Therefore the isomers of Si_n^+ and C_m^+ have two structural motifs ; spherical and non-spherical structures. These different structures may show marked difference in their reactivity. Therefore the isomer-resolved measurement is necessary in order to examine the reactivities and reaction mechanism rigorously. Ion mobility spectrometry, IMS, is a powerful tool to directly separate isomers. However, the ion intensity drops by several orders of magnitude after the isomer-separation with this method. It is also important to obtain spatially focused ion bunches for unambiguous and highly-sensitive photodissociation measurements of size-selected ions.

In order to fulfill the isomer-resolved photodissociation experiments against above problems, we have developed a combined setup of an ion drift cell with a double reflectron time-of-flight mass spectrometer, TOFMS. In this setup, dissociation efficiency is enhanced by the focusing of parent ions at the irradiation point after a first reflectron. In this study, we have examined UV laser photodissociation reactions for each isomer of $Si_n^+$and C_m^+ after mass- and isomer-separations. Si_n^+ and C_m^+ generated by laser vaporization were isomer-separated by a drift cell, in which the ions were collided with He under an electrostatic field. The cell was cooled to 170 K. The separated isomers were mass-separated in a double reflectron TOFMS setup. The ions were then dissociated by irradiation with a 4.66 eV laser at the ion focal plane in the first reflectron. The photofragment ions were mass-analyzed by the second reflectron.

In the isomer-separated experiments for the parent Si_n^+ions with n = 24-27, we have observed two types of fragment ions, $Si_{6\text{-}11}^+$ fragment clusters and Si_{10}-loss fragment ions, from both the prolate and spherical isomers. In particular, relative intensities of Si_{10}-loss fragments from the prolate isomers were found to be slightly higher than that from the sphericals. This result was partly explained from the calculated geometric structures containing Si_{10} units [1] .

On the other hand, forC_m^+ (m = 32, 34, 36, 38) , we have observed clearly different fragmentation pathways between the cyclic and fullerene isomers ; C_{14}-loss was preferentially observed from the cyclic isomers, whereas sequential C_2-losses were obtained from the fullerenes. The C_{14}-loss products were already reported in dissociation studies of C_m^+ with $m< 29$, where stable isomers have multi-cyclic structures [2] . This predominant pathway was explained by the neutral C_{14} stability based on the Hueckel model. The C_2-loss pathways of fullerenes are also consistent with the previous photodissociation studies on fullerenes [3] .

[1] S. Yoo and X. C. Zeng, *J. Chem. Phys*. **124**, 054304 (2006) .

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Poster Session

Monday, 17th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

PMo-001

11:10 – 12:20

Boundary Activated Dissociation in a Low Pressure Linear Ion Trap in the presence of Nonlinear DC Fields

mircea guna

AB SCIEX, Concord, ON, Canada

Keywords:
BAD, CAD, nonlinear fields

Novel aspects:
Nonlinear dc fields modify the stability boundary of the quadrupole, increasing the BAD efficiency to 94% and extending the mass range of the BAD fragmentation spectrum beyond previously observed limits.

Abstract:
Boundary-Activated Dissociation (BAD) of multiple charge ions has been investigated in a low pressure linear ion trap in the presence of nonlinear dc fields. Nonlinear dc fields allowed ions to be stored at working points beyond the by= 0 stability boundary of the regular quadrupole. The ions reached large stable radial amplitude trajectories and gained high kinetic energies from the main drive RF field and thus the BAD efficiency increased from less than 20% to upto 94%. The broadening of the stability diagram at the by = 0 boundary also enabled the observation of fragment ions of higher mass-to-charge ratio than the precursor ions thus overcoming one of the major drawbacks of BAD of multiple-charged ions. A comparison between high pressure beam-type collision activated dissociation (CAD) and low pressure BAD spectra showed that similar internal energies can be deposited using these two techniques however less fragmentation channels were observed using low pressure BAD.

PMo-002
13:30 – 14:40

ECD in an RF-Free Electromagnetostatic Cell on a Triple Quadrupole Mass Spectrometer

Yu Chu Lin[1], Samuel Bennett[1], Valery G Voinov[1,2], Douglas F Barofsky[1]

[1]Oregon State University, Corvallis, Oregon, U.S.A., [2]Pacific Institute of Bioorganic Chemistry, Vladivostok, Rusia

Keywords:
Electron Capture Dissociation (ECD) ; Ion Activation/Dissociation ; Electromagnetostatic dissociation cell

Novel aspects:
Enhanced ECD in a radio-frequency-free electro-magnetostatic cell

Abstract:

Introduction

Last years we demonstrated a Radio Frequency-Free (RFF) electro-magnetostatic (EMS) cell for producing electron capture dissociation without the aid of a cooling gas or phase-specific electron injection into the cell as part of a triple quad and a hybrid Q-TOF tandem mass spectrometer [1-3]. That work further demonstrated not only ECD, but also combined ECD and collision-induced dissociation (CID) in an RFF EMS cell [4]. In this presentation, we describe an improved EMS cell design and present results that demonstrate that this improved cell's ECD efficiency is at the threshold of analytical usefulness.

Methods

A triple quadrupole (Q-q-Q) Finnigan TSQ 700 mass spectrometer was converted to a Q-ECD-Q instrument :

·An indirectly heated, ring-shaped, Tantalum disc (Heat Wave Labs, Inc., Watsonville, CA) ,located concentric with the cell's axis at the ion-entrance, serves as the source of electrons.·

The cell comprises an electromagnet and anaxially polarized Sm_2Co_{17} ring-magnet (Chino Magnetism Corp. Ltd., Fairfield, NJ) that has a 12.54 mm diameter, 1.0 mm thickness, and 2.0 mm bore. Several common peptides were introduced into the instrument by ESI in order to produce parent ions for the ECD experiments.

Preliminary Data

Using an indirectly heated disc as a source of electrons instead of a directly heated, ring-shaped, wire filament solved three important problems. First, the greatly increased emitting area of the disc over that of the wire filament emitted a much larger number of electrons. Second, the problem of voltage drop through the emitter was eliminated ; in the case of the ring-shaped, wire filament, this voltage drop could be up to 7 V resulting in an excessively wide electron energy distribution. Third, the problem of the magnetic field induced by the current in the ring-shape wire filament negatively effecting electron emission was totally eliminated. Taken together, the gains resulting from these three improvements increased the ECD efficiency of EMS cell up to at least 2 %, which is at the threshold of analytical utility. As example, ECD fragments recorded while injecting 5 μg/mL solution of substance P in methanol at a flow rate of 2 μL/min are present.

Summary

In summary, we have achieved ECD in a linear, RF-free, hybrid electrostatic/magnetostatic cell at efficiency level of at least 2 % without the aid of a cooling gas. The cell's design and compact construction allow it to be incorporated into virtually any type of tandem mass spectrometer, e.g., triple quadrupole, hybrid quadrupole ion trap, hybrid quadrupole time-of-flight, or even FT-ICR.

Acknowledgement

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PMo-003

11:10 – 12:20

A rapid and sensitive liquid chromatography tandem mass spectrometry method for quantification of etoposide and etoposide chatechol in cerebrospinal fluid

Hsiao-Wei Liao[1,2], Yen-Shen Lu[3], Ching-Hung Lin[3], Ching-Hua Kuo[1,2]

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Keywords:
Etoposide, liquid chromatography tandem mass spectrometry, cerebrospinal fluid

Novel aspects:
This is the first time using liquid chromatography tandem mass spectrometry method for quantification of etoposide and etoposide catechol in cerebrospinal fluid.

Abstract:
Cancer with brain metastasis is one of the leading causes of death in many major cancer subtypes. Etoposide is one of the chemotherapy drugs that can partially penetrate blood brain barrier. In human body, etoposide undergoes O-demethylation and can be metabolized into etoposide catechol which exhibits signicant cytotoxic activity. Understanding the etoposide and etoposide chatechol concentration in CSF for patients with brain tumor can help to evaluate the drug efficacy. A rapid and sensitive ultra-high-pressure liquid chromatography/electrospray ionization tandem mass spectrometry (UHPLC-MS/MS) method was developed for quantification of etoposide and etoposide chatechol in CSF.

Chromatographic separation was performed on an Agilent Extend RP-18 column (1.8 um, 50 mm×2.1 mm) with a gradient mobile phase consisted of 0.1 % acetic acid in water and methanol delivered at 0.3 ml/min. Detection was achieved by an Agilent 6460 UHPLC-MS/MS system with selected reaction monitoring (SRM) in positive ion mode with the transition m/z 589.6/229.1 and m/z 575.2/229.0 for etoposide and etoposide chatechol, respectively. The limit of detection (LOD) is 0.5 ng/mL and the limit of quantification (LOQ) is 1 ng/mL for both etoposide and etoposide chatechol. The total analysis time is below 3 minutes. The method was validated in terms of precision, accuracy, matrix effect and linearity. Finally, this method was successfully applied to the analysis of CSF samples from cancer patients with brain and leptomeningeal metastasis treated with etoposide, and the pharmacokinetic behavior of etoposide in CSF was determined.

Poster Session

Monday, 17th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

PMo-004
13:30 – 14:40

Qualitative analysis of impurities in enalapril using liquid chromatography-ion trap time of flight hybrid mass spectrometry

FENG JI, QI Y LI, JING DONG
Shimdzu Co., Beijing,China

Keywords:
Enalapril, Impurities, LCMS-IT-TOF

Novel aspects:
To our knowledge, this is the first complete characterization of the fragmentation of enalapril impurities, mainly are its by-products, using ESI-IT-TOF tandem mass spectrometry with accurate mass measurements.

Abstract:
Enalapril Maleate is the maleate salt of enalapril, the ethyl ester of a long acting angiotensin converting enzyme inhibitor. Enalapril is a pro-drug ; it is bioactivated by hydrolysis of the ethyl ester to enalaprilat, which is the active angiotensin conveting enzyme inhibitor. Enalapril has side effects : fainting ; urinating more ; chest pain, etc. However, by-products, unknown impurities and their structures are a big concern. Currently, high-performance liquid chromatography (HPLC) has been the essential method for analysis of enalapril. Atmospheric pressure ionization sources, such as electrospray ionization (ESI) are routinely used in drug impurities analysis for sensitivity.

A liquid chromatography/electrospray ion trap time-of-flight mass spectrometry (LCMS-IT-TOF) was used in this study. It has the ability to provide multistage tandem spectra (MS^n) with accurate masses (error< 5 ppm) in both MS and MS^n modes. Tof analyzer allows fast acquisition of full spectra with high sensitivity and elevated mass resolution (12000 FWHM) . With this technique, we can acquire reliable structural information about the ionized molecule and the product ions, based on the exact mass and the multistage tandem mass spectrometric analyses (MS^n) . In addition, as the mass/charge ratio gets smaller, the number of candidates gets smaller, too. Since mass/charge ratios of the product ions in the highest order spectra are usually less than 200 Da and the neutral losses are usually less than 100 Da, their formulae could be confirmed with significantly higher confidence.

Enalapril samples were separated on a Shimadzu Shim-pack XR-ODS column (2.0 mm I.D.×75 mm L., 2.2 μm) with isocratic elution using 10 mM ammonium acetate and acetonitrile as mobile phase. High concentration of enalapril (300 mg/L) was injected to concentrate impurities. Flow path switching valve was used in the instrument, cutting the enalapril main constituent to waste line in order not to contaminate MS detector. Four impurities were separated and detected by ESI-IT/TOF. MS^n spectra (n=4) of each impurity were obtained in both positive and negative ion mode. The combination of accurate mass measurement in MS spectra and sequential MS^n experiments enabled fragmentation pathways to be elucidated in detail. Formulae, chemical structures of impurities in an Enalapril sample were suggested with supporting results on the probable fragmentation pathways.

Protonated molecules of impurity 1 (m/z 349.1758) gave product ions at m/z 303.1703 with a neutral loss of m/z 46.0055, which was CH_2O_2. Using formula predict software impurity 1 was $C_{18}H_{24}N_2O_5$. Similar MS^n measurement was observed between impurity 1 and enalapril. This could be explained by loss of two methylene group (perhaps carboxylic acid ethyl ester) from enalapril structure.

Formula predict of impurity 2 m/z 381.2395 shows a formula as $C_{20}H_{34}N_2O_5$,contains 6 more H atom compared to enalapril. When m/z 381 was selected as the precursor ion to perform the MS^2 product ion scan experiment, a product ion at m/z 335.1976, 307.2027, 291.2078, 273.1972, 263.2129 and 192.1394, all have corresponding response loss of 6 H in enalapril. Product ion at m/z 183.0775,170.0823 were both observed at impurity 2 and enalapril. m/z 183.0775,170.0823 is formed by the loss of benzene ring form enalapril. So the structure of impurities 2 may be explained by deoxidation of benzene ring from enalapril.

Also we may get the formulae for impurity 3 and 4 are $C_{20}H_{26}N_2O_4$ and $C_{19}H_{26}N_2O_4$. To our knowledge, this is the first complete characterization of the fragmentation of enalapril impurities, mainly are its by-products, using ESI-IT-TOF tandem mass spectrometry with accurate mass measurements.

PMo-005

11:10 – 12:20

Qualitative Analysis of Gossypol, Free Gossypol, and Gossypol Derivatives in Cottonseeds By Electrospray Ionization Tandem Mass Spectrometry

Luying Zhou[1], Lai Chen[2], Jinting Yao[1], Hongyuan Hao[1], Hengtao Dong[1], Qiang Li[1]

[1]Shimadzu (China) Co., Ltd., Shanghai, China, [2]Shanghai Ocean University

Keywords:
Gossypol Derivatives, LCMS-IT-TOF, MSn Spectra

Novel aspects:
The study of derivatives struture by MSn Spectras got from LCMS-IT-TOF

Abstract:
Free gossypol and in cottonseed is a toxic chemical, which can affect human reproductive function, skin and stomach burning, nausea, vomiting, diarrhea, headache, coma, convulsions and even death due to respiratory, circulatory system failure. Therefore, strict control of the free gossypol and its derivatives is required in deep-processing process of cottonseed. In this article, according to the MS^n Spectra of gossypol, the structures of known and unknown gossypol derivatives were figure out by LCMS-IT-TOF. Quantity and quality of free gossypol derivatives were different when using different extracting way. The qualitative results of gossypol derivatives get from LCMS-IT-TOF could tell the best way of deep-processing process of cottonseed.

Poster Session

Monday, 17th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

PMo-006
13:30 – 14:40

Evaluation of the higher sensitive LC/MS/MS incorporates novel desolvation technologies to achieve low femto-gram LOQ

Shinjiro Fujita, Natsuyo Asano, Kazuo Mukaibatake
Shimadzu Corporation, Kyoto, Japan

Keywords:
LC/MS/MS, desolvation, heated gas, off axis, orthogonal

Novel aspects:
Low femto-gram LOQs were achieved by using highly sensitive LC/MS/MS with heated gas and multi orthogonal heated device

Abstract:
The triple quadrupole mass spectrometer is widely used in various application fields to quantify the trace amount of compounds in real world samples because of its excellent sensitivity and selectivity. In order to achieve lower limit of quantitation, many researchers have been developing new desolvation technologies at either ESI sprayer or MS inlet.
Here, we report the higher sensitive triple quadrupole mass spectrometer equipped with novel desolvation devices such as addition of the heated gas blowing along with electrospray and the heated multi-orthogonal ion path mounted at the inlet of mass spectrometer. The combination of the heated gas at ESI and heated multi-orthogonal ion path enhanced desolvation efficiency which resulted in low femto-gram limit of quantitation.
Several commercially available drug samples (Reserpine, Verapamil, Alprazolam, Carbamazepine, Cilostazol, Lidocaine, Fluticasone and Testosterone) were prepared for the sensitivity evaluation of higher sensitive triple quadrupole mass spectrometer equipped with the modified ESI source and the heated multi orthogonal ion path. All the samples were analysed by the Multiple Reaction Monitoring (MRM) . MRM parameters including MRM transitions and collision energy as well as compound dependent ion transfer voltages were optimised through automatic MRM optimization functionality incorporated in LabSolution software (Shimadzu Corporation, Kyoto, Japan) .The temperatures of heated gas and heated multi-orthogonal ion path were optimised for each sample.
Using modified ESI source and the heated multi orthogonal ion path helped to deliver a limit of quantitation for all eight drugs below 5 fg/uL. The temperature of heated gas and heated multi-orthogonal ion path were independently optimised for each sample with the range of 200 to 500 degree C and 150 to 300 degree C respectively. The ion intensity of reserpine was increased 8 times with the heated gas and 4 times with heated multi-orthogonal ion path operated at 300 degree C. Besides larger volume of Ion was introduced into the mass spectrometer by the effective desolvation, the base line noise was maintained at reasonably low level as the noise derived from neutral droplet or unwanted ions were strongly reduced in the multi orthogonal region heated at high temperature, and resulted in excellent signal to noise ratio which achieved low femto-gram sensitivity.

PMo-007
11:10 – 12:20

Determination and pharmacokinetics of di-(2-ethylhexyl) phthalate in rats by ultra performance liquid chromatography with tandem mass spectrometry

Wan-Ling Chang-Liao[1], Lie-Chwen Lin[1,2], Tung-Hu Tsai[1,3]

[1]National Yang-Ming University, Taipei, Taiwan, [2]National Research Insitute of Chinese Medicine, [3]Taipei City Hospital, Taipei, Taiwan

Keywords:
bioavailability, Di- (2-ethylhexyl) phthalate (DEHP), pharmacokinetics, plasticizer, tandem mass spectrometry.

Novel aspects:
This is the first time that a LC-MS/MS has been developed to investigate oral bioavailability in rat.

Abstract:
Di- (2-ethylhexyl) phthalate (DEHP) is generally used to increase the flexibility of plastics for industrial products which has been contaminated in food and beverages as clouding agent in Taiwan in the early 2011. Previous studies indicated that DEHP may lead to hepatotoxic, cytotoxic, teratogenic and mutagenic effects on animals. In order to assess the exact extent of absorbed DEHP via the oral route, the aim of this study is to develop a reliable and validated ultra performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) method for the evaluation of the oral bioavailability of DEHP in Sprague-Dawley rats. The optimal chromatographic separation of DEHP and Butylbenzyl phthalate (BBP ; as internal standard) were achieved on a C_{18} column (2.1 mm x 100 mm, 1.7 μm) . The mobile phase consisted of 5 mM ammonium acetate-methanol (11 : 89, v/v) with a flow rate of 0.25 mL/min. The monitoring ion transitions were m/z 391.4 → 149.0 for DEHP and m/z 313.3 → 149.0 for the internal standard. The average of matrix effect and recovery of DEHP at low, medium and high concentrations was 113 ± 20% and 98 ± 5 %. The calibration curve is linear within the given concentration range from 0.1-2.5 μg/mL and produced linear correlation coefficient is greater than 0.995. In conclusion, the validated UPLC-MS/MS method is suitable for analyzing the rat plasma sample of DEHP and the oral bioavailability of DEHP was about 7 % in rats.

PMo-008
13:30 – 14:40

Pharmacokinetics of Di-isononyl Phthalate in Freely Moving Rats by UPLC-MS/MS

Mei-Ling Hou[1], Tung-Hu Tsai[1,2]

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Keywords:
DINP, phthalate, freely moving rat, pharmacokinetics, tandem mass spectrometry

Novel aspects:
This is the first time that a LC-MS/MS has been developed to investigate oral bioavailability of DINP in rat.

Abstract:
Di-isononyl phthalate (DINP) is a general-purpose plasticizer for polyvinyl chloride. However, the industrial chemical plasticizer had contaminated food and beverages as clouding agent, which had been inspected by Taiwan Food and Drugs Administration. The aim of study is to develop a sensitive and specific ultra-high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-MS/MS) method to investigate the pharmacokinetics of DINP in freely moving rats. Multiple reaction monitoring (MRM) was used to monitor the transition of the protonated molecule m/z of 419 $[M+H]^+$ to the product ion 149 for DINP. Plasma and feces homogenates were extracted by protein precipitation with 100 % acetonitrile (0.1% formic acid) for sample preparation. The analyte was analyzed by UPLC-MS/MS with C18 column (100 mm x 2.1 mm, 1.7 µm) which was equilibrated and eluted with an isocratic mixture of acetonitrile-ammonium acetate water solution (90 : 10, v/v) at a flow rate of 0.3 mL/min. The limit of quantification (LOQ) of DINP in rat plasma and feces homogenate was determined to be 0.25 µg/mL. The intra- and inter-day accuracy (% Bias) and precision (% CV) were less than 15 %. Linear calibration curves were obtained for DINP concentration ranges of 0.05-2.5 µg/mL in plasma and feces. The feces were homogenized mechanically using 50 % acetonitrile as the media. The pharmacokinetic curve demonstrated that the disposition of DINP in rat plasma was fitted well by the two-compartmental model after DINP administration (10 mg/kg, i.v.) . The elimination half-life of DINP was 5.87 ± 3.41 and 150.41 ± 58.38 min for intravenous (10 mg/kg) and oral (100 mg/kg) administration, respectively. The pharmacokinetic data indicate that the oral bioavailability of DINP in freely moving rats was about 1.19 %. The total DINP excretion up to 48 h was 12.30 ± 4.46 % in feces.

PMo-009
11:10 – 12:20

Pharmacokinetics of dibutyl phthalate by ultra performance liquid chromatography with tandem mass spectrometry in rats

Li-Wen Chang[1], Tung-Hu Tsai[1,2]

[1]National Yang-Ming University, Taipei, Taiwan, [2]Taipei City Hospital, Taipei, Taiwan

Keywords:
dibutyl phthalate, pharmacokinetics, plasticizer, tandem mass spectrometry.

Novel aspects:
This is the first time that a LC-MS/MS has been developed to investigate pharmacokinetics of DBP in rat.

Abstract:
Dibutyl phthalate (DBP) is a commonly used plasticizer for flexible polyvinyl chloride with a broad range of applications. However several plasticizers have been illegally used as clouding agent to form dispersion in aqueous matrix of the drinks. The aim of study is to establish a rapid and accurate analytical method to investigate the pharmacokinetics of DBP in Sprague-Dawley rat by Ultra Performance Liquid Chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) . The UPLC-MS/MS system equipped with a positive electrospray ionization (ESI) source in multiple reaction monitoring (MRM) mode was used to monitor m/z 279.28 → 148.93 and m/z 225.09 → 102.96 transitions for DBP and chrysin (internal standard) , respectively. The analytes were separated using a C_{18} reversed-phase column (2.1 mm x 100 mm, 1.7 μm) , the mobile phase for column separation was 5 mM amonium acetate mthanol (27 : 73, v/v) with a flow rate of 0.25 ml/min. The method has been applied to measure DBP in rat plasma and faece by protein precipitation procedure. The limit of quantification for DBP in rat plasma and faese was 50 ng/ml and 125 ng/ml, respectively. The pharmacokinetic results demonstrated that the maximum concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) in rat plasma were 2.88±0.17 μg/mL and 7.11±0.50 min, respectively after DBP administration (30 mg/kg, i.v.) . About 0.9 % of the administered dose was recovered from the faece within 48 h. The results showed that DBP degraded within 2 h, suggesting a rapid metabolism of DBP in the body.

Poster Session

Monday, 17th September — Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 1: Developments in Tandem Mass Spectrometry - Hybrid Instrumentation "The whole is greater than the sum of its parts" (Aristotle).

PMo-010
13:30 – 14:40

Separation of polar solutes by hydrophilic methacrylate-based monolithic columns via capillary electrochromatography coupled with UV-Vis and mass spectrometry detection.

Yung-Han Shih, Ching-Yi Wu, Cheng-Lan Lin, Pei-Zhu Liang, Hsi-Ya Huang
Chung Yuan Christian University, ChungLi, Taiwan

Keywords:
hydrophilic organic polymer-based monolith ; capillary electrochromatography ; CEC-MS ; phenolic acid ; amino acid

Novel aspects:
This study demonstrated a novel poly (TAIC-EDMA-SMA) monoliths which are highly potential stationary phases for hydrophilic solutes separation.

Abstract:
Abstract
In traditional methacrylate ester-based monoliths, it is very hard to separate polar compounds. In this study, we describe triallyl isocyanurate as hydrophilic cross-linker to synthesize poly methacrylate ester monolithic columns for the separation of polar solutes in capillary electrochromatography (CEC) . Different ratios of triallyl isocyanurate to ethylene dimethacrylate (EDMA) and alkyl methacrylates were optimized and characterized by SEM to evaluate the morphology, N_2 adsorption/desorption isotherm to determine the surface area and thermogravimetric analysis for thermal stability. Compared with traditional methacrylate ester-based monoliths, the triallyl isocyanurate-co-methacrylate monoliths exhibit hydrophilicity, better thermal stability and good baseline separation ability for all polar analytes at a mobile phase composed of 18~27.5 % acetonitrile. Moreover, these triallyl isocyanurate-co-methacrylate columns were also applied in CEC-ESI/MS.

PMo-011
11:10 – 12:20

The COrrelation Ion-Neutral Time Of Flight Mass Spectrometry (COIN-TOF_MS)

Victor Buridon[1], Cecile Teyssier[1], Hassan Abdoul-Carime[1], Bernadette Farizon[1], Michel Farizon[1], Tilmann D Mark[2]

[1]Universite de Lyon 1, Institut de Physique Nucleaire Lyon, Villeurbanne, France, [2]Institut fur Ionenphysik und Angewandte Physik, Leopold Franzens Universitat, Technikerstrasse 25, A-6020 Innsbruck, Austria

Keywords:
Ion-Neutral correlation, event-by-event analysis technique

Novel aspects:
COIN-TOF MS as a new and versatile instrument for the investigation of collision processes and analytical chemistry

Abstract:
Mass-spectrometry is nowadays widely used not only for the analysis of (bio-) chemistry products [1] but also for the investigation of processes (e.g., ion, electron or photon-nano-system collisions) [2] . Collecting and correlating all the nascent fragments produced in a given dissociation pathway represent a tremendous challenge for an accurate characterization of the parent molecule or the description of the interaction mechanisms. The mass of charged products can be determined with high accuracy by time-of-flight mass spectrometry [3] . In contrast, ascribing the neutral species requires very specific conditions [4] . The coincidence measurements between sets of fragments may be obtained only by using multi-detectors technique [5] , however the correlation between fragments within a given dissociation event are still not accessible. These become even more challenging for complex nano-systems when branching ratios between several competitive channels for neutrals production are opened.
We present the COIN-TOF Mass Spectrometer [6] . This new and versatile instrument is based on a single detector for the detection of the ion and the neutral fragments produced within a single dissociation event and the "event-by-event " analysis of data. Such analysis provides information on the ion and neutral products, their correlations for a given dissociative channel and also the nature of the prior interaction that leads to the dissociation of the investigated molecular system. Thus, physical parameters i.e., branching ratios for neutral fragments, kinetic energy releases., can be directly accessible. Illustration of the COIN-TOF_MS will be discussed through selected examples obtained from recent collision induced dissociation measurements [7] .

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PMo-012
13:30 – 14:40

Automatic Disulfide Bond Assignment Using a1 Ion Screening by Mass Spectrometry for Structural Characterization of Protein Pharmaceuticals

Sheng-Yu Huang[2], Yu-Ting Hsieh[1], Chun-Hao Chen[1], Chao-Chi Chen[2], Wang-Chou Sung[3], Min-Yuan Chou[4], Sung-Fang Chen[1]

[1]National Taiwan Normal University, Taipei, Taiwan, [2]Mithra Biotechnology Inc., Taiwan, [3]National Health Research Institutes, Taiwan, [4]Industrial Technology Research Institute, Taiwan

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Keywords:
disulfide bonds, dimethyl labeling, pharmaceuticals

Novel aspects:
An automatic method for disulfide bond assignment using dimethyl labeling and computational screening of a 1 ions by MS analysis with customized software, RADAR, is developed.

Abstract:
An automatic method for disulfide bond assignment using dimethyl labeling and computational screening of a_1 ions by MS analysis with customized software, RADAR, is developed. Utilizing the enhanced a_1 ions generated from labeled peptides, the N-terminal amino acids from disulfide-linked peptides can be determined. In this study, we applied this method for structural characterization of recombinant monoclonal antibodies, an important group of therapeutic proteins. In addition to a_1 ion screening and molecular weight match, new RADAR is capable of confirming the matched peptide pairs by further comparing the CID fragment ions. With the N-terminal amino acid identities as a threshold, the identification of disulfide-linked peptide pairs can be achieved rapidly at a higher confidence level. Unlike majorities of the current approaches, prior knowledge of disulfide linkages or a high- end mass spectrometry is not required, and tedious labor work or deliberate interpretation can be avoided in this study. Our approach makes it possible to analyze unknown disulfide bonds of protein pharmaceuticals as well as their degraded forms without further protein separation. It can be used as a convenient quality examination tool during biopharmaceutical development and manufacturing processes.

PMo-013
11:10 – 12:20

Determination of chloramphenicol in biological samples by gas chromatography-mass spectrometry

Suh-Jen J Tsai, Hsiu-Hua Huang, Pei-Yin Hsieh, Che-Wei Chang

Providence University, Taichung, Taiwan

Keywords:
Chloramphenicol ; Derivatization ; GC-MS ; Biological samples

Novel aspects:
An analytical method has been established for the determination of chloramphenicol (CAP) . The derivatization of non-volatile CAP with either N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) or N- (tert-buthyldimethylsilyl) - N-methyltrifluoroacetamide (MTBSTFA) reduced the polarity of CAP.

Abstract:
Determination of **c**hloramphenicol in biological samples by gas chromatography-mass spectrometry

Suh-Jen Jane Tsai*, Hsiu-Hua Huang, Pei-Yin Hsieh, Che-Wei Chang

Department of Applied Chemistry, Providence University, Taichung , Taiwan 43301
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Abstract
An analytical method has been established for the determination of chloramphenicol (CAP) . CAP is one of the most important antibacterials. CAP has often been used in the animal and fish farms to prevent diseases, to fight parasites, and to promote growth. GC-MS is less expansive then LC-MS, and consequently, is more popular in the routine laboratories. However, it is not applicable to analysis the non-volatile CAP directly by GC-MS. The derivatization of non-volatile CAP with either N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) orN- (*tert*-buthyldimethylsilyl) - N-methyltrifluoroacetamide (MTBSTFA) reduced the polarity of CAP. The derivatives were effectively determined by GC-MS. TThe experimental conditions for GC-EI-MS were optimized. These included the optimum injection temperature, injection volume for the derivatization condition, reaction solvent, and reaction time...etc. GC-EI-MS was done with extracted ion mode. The derivatization efficiency of silation and acetylation were compared. The accuracy and precision of this proposed method for the determination of chloramphenicol in biological samples including pig liver, pig kidney, and Bovine liver were also evaluated.

PMo-014
13:30 – 14:40

Combining quantum mechanical and MS analysis to understanding the source of H+ during APPI of PAH dissolved in toluene

Arif Ahmed, Chell Ho Choi, Kim Sunghwan

Kyungpook National University, Daegu, Korea

Keywords:
quantum mechanics, appi, mass spectromery

Novel aspects:
Mechanism of APPI of studied by combining experimental MS data and quantum mechanical calculation

Abstract:
Atmospheric pressure photo ionization (APPI) is a powerful technique for analysis of polycyclic aromatic compounds (PAH) which are very important in environmental and crud analysis. In a previous study published in our group, it has been shown that toluene is the source H^+ for APPI when only toluene was used as a solvent. In this study, APPI mechanism, especially, the source of H^+ during the generation of protonated PAH compounds were studied by quantum mechanical calculation and mass spectrometry analysis.

Antharacene, acridine, HPLC grade toluene, water, MeOH, anisole and benzene were purchased from Sigma-Aldrich (MO, USA) and used without further purification. Standard samples were dissolved in toluene, anisole and benzene solvent to final concentration of 1 μM~10 μM. The solutions were analyzed by positive mode APPI LCQ Fleet ion trap mass spectrometer (Thermo Scientific) . The General Atomic and Molecular Electronic Structure System (GAMESS) program was used for all of the computations. Density functional theory with B 3 LYP functionals was utilized in combination with an all-electron 6 -31G (d,p) basis set. Minimum energy reaction paths were determined by first optimizing the geometries of the minima and transition states.

Anthracene was dissolved in toluene, anisole and benzene and analyzed by APPI MS. Only anthracene dissolved in toluene produced protonated ions. This strongly suggests that the hydrogen in methyl group of toluene molecules but not the one in the aromatic ring is used to produce protonated ion. Additionally, APPI MS spectra of acridine dissolved in toluene, anisole and benzene were obtained and compared. It was observed that all three combinations of anthracene-toluene, anthracene-benzene and anthracene-anisole dominantly produced protonated ion. The data suggest that both hydrogen in methyl group and aromatic ring can be used to produce protonated ions for acridine. To further study the findings, theoretical calculation is currently undergoing. The general atomic and molecular electronic structure system (GAMESS) program is being used for computations.

PMo-015
11:10 – 12:20

Transformation of the Green Fluorescence Protein upon transfer from solution to the gas phase.

Vladimir Frankevich, Konstantin Barylyuk, Paviel Sagulenko, Renato Zenobi

Swiss Federal Institute of Technology, Zurich, Switzerland

Keywords:
Fluorescence, GFP, FT-ICR, spectroscopy

Novel aspects:
GFP conformation in the gas phase. Novel method to probe the properties of gas-phase molecular ions

Abstract:
The combination of laser-induced fluorescence (LIF) with mass spectrometry opens up new possibilities for structural studies of biomolecular ions in the gas phase. What will happen to a protein when the solvent molecules are removed? Will it maintain a near-native conformation, unfold, or assume a new structure? The green fluorescent protein (GFP) is highly fluorescent in its native conformation, while it does not fluoresce upon unfolding. Observing the fluorescence of GFP in the gas phase can therefore serve a very direct measure of its conformation.

A Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS) coupled with laser spectroscopy was chosen as an experimental platform to probe the properties of isolated biomolecular ions. A unique home-built optical setup was constructed that allows laser-induced fluorescence/dissociation experiments on trapped ions in the gas phase. Ions can be produced externally by electrospray ionization (ESI) and trapped for high resolution mass analysis and spectroscopic studies in the FTICR cell.

We performed a series of laser spectroscopy experiments in order to probe the conformation of gas-phase GFP ions directly inside the ESI plume and when trapped in the high vacuum of the FTICR mass spectrometer. GFP fluorescence spectra in the ESI source were found to be unchanged along the plume, but the signal intensity gradually decreased. In additional experiments, a trapped GFP ion population was subjected to CW laser irradiation (from 351 nm to 514 nm) of various powers (up to 1.5 W) and exposure times (up to 2 min) . Interestingly, neither fluorescence nor dissociation was observed for trapped GFP ions. On the contrary, synthetic cR6G labeled protein-dye conjugates prepared from ubiquitin and GFP showed fluorescence signal when isolated in the FTICR cell under same conditions. The lack of fluorescence from GFP could be directly attributed to protein unfolding in vacuum. On the other hand, the fluorescence could disappear due to the loss of structural water molecules. Water molecules are important contributors to the stability and functioning of biomolecules. There is evidence that the structural water participates in the GFP fluorescence process. Molecules of structural water build a hydrogen bond network that is necessary for GFP fluorescence. Our high-resolution MS experiments show the complete absence of structural water molecules in GFP. This does not affect the GFP conformation too much, but dramatically changes its fluorescence properties. Our preliminary data suggest that this is the mechanism responsible for the lack of GFP fluorescence in vacuum.

PMo-016
13:30 – 14:40

Elucidation of CID and ETD based MS/MS Fragmentation for Analysis of Doubly Derivatized Steroids

Yu-Min Juang, Tzu-Fang She, Chien-Chen Lai

Institute of Molecular Biology,National Chung Hsing University, Taichung, Taiwan

Keywords:
Steroids, ETD, Microwave-accelerated derivatization

Novel aspects:
ETD conjunction with CID supplied more structure information of doubly derivatized steroids, and expected that a novel and sensitive metohd could be applied for steroid analysis.

Abstract:
The specific measurements of target steroids play an important role in the clinical diagnosing of a number of common endocrine disorders and testing drug abuse in athletes. More recently, liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) with collision-induced dissociation (CID) has advantage that detection of complex sample and time-reduced of pretreatment, and it is a rapid and convenient technique for examination of disease and drugs. Nevertheless, steroid assay is difficult to develop specific methods for the detection of an individual steroid in the presence of many structurally similar compounds. To further develop a specific method with sensitivity and precision, we conducted a complementary MS/MS analysis of doubly Girard's reagent P derivatized steroids by using combined CID and electron transfer dissociation (ETD) . This approach proved that derivatization with Girard's reagent P by microwave irradiation led to higher ESI sensitivity of steroids and reduces the reaction time less than 10 min. As the result of derivatized steroids underwent two fragmentation methods, CID provided much product ions which are low m/z and the fragment mechanism was random and some was perplexity. However, ETD provided high m/z ions and sequential fragment mechanism which was easy to recognize. These findings indicate that the analysis of doubly derivatized steroids by ETD in conjunction with CID can provide more structural information of B- and C-ring, and with side chain on it. Furthermore, establishment of both CID and ETD fragment ions database that from nine doubly derivatized steroids expected to improve steroid identification.

PMo-018 Identification of protein ligands by FT-ICR-MS/MS

13:30 – 14:40

Adam A Dowle, Abbas Maqbool, Gavin H Thomas, Jerry Thomas

University of York

Keywords:
FT-MS/MS SORI CID Bruker solariX ligand

Novel aspects:
Protein ligands were identified by FT-ICR-MS/MS using CID and SORI with a Bruker solariX, which allowed revision of proposed metabolic pathways.

Abstract:
The presence of non-covalently bound ligands in proteins can have a variety of significant biological effects ; examples are numerous including biological synthesis, cell signalling, and transport. Accurate identification of the ligands involved is of vital importance in these processes. However, conventional mass spectrometry and even X-ray crystallography cannot always provide definitive identification, especially where sample is limited in amount. The high mass accuracy of FT-ICR-MS, in tandem with its ability to couple with a variety of ion dissociation options, offers good scope for the identification of these biological molecules. We have demonstrated that the Bruker solariX equipped with a 9.4-tesla magnet can be used to accurately identify non-covalent protein ligands.

Ligand identification by FT-ICR-MS was performed under denaturing conditions to maximise signal quality and mass accuracy. Acquisition parameters were adjusted to aid release of the ligand and optimise sensitivity in the low *m/z* region where the ligands are found. Important acquisition parameter changes included increasing the source accumulation time and reducing the TOF time. Typically, identification of elemental composition was possible from the intact *m/z* alone, with indications of structure arising from fragmenting the ligand using different dissociation methods. The solariX affords a variety of dissociation options ; so far we have performed fragmentation of ligands using CID in the quadrupole and SORI in the Infinity™ cell. These approaches have yielded differing and complementary product ion spectra that aid the assignment of ligand structure. In the case of L-alanyl- γ -D-glutamyl-mesodiaminopimelate the y_1-ion pedominated in the SORI spectrum while the y_2-ion was only detected following CID. Example of ligands identified include : L-alanyl- γ -D-glutamyl-mesodiaminopimelate ; glutathione ; sialyl amide and S-adenosyl, S-carboxymethylhomocysteine. Identifications were consistent with results of structural binding assays or X-ray crystallography data.

PMo-019
11:10 – 12:20

Absolute Internal Mass Calibration with Carbon Soot Ions for High Resolution MALDI 15T FT-ICR Mass Spectrometry

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Keywords:
Mass Calibration, Carbon Soot, FTICR MS, 15T

Novel aspects:
With the high resolution carbon cluster peaks, the mass accuracy of internal calibration was improved in 15 T MALDI FT-ICR MS.

Abstract:

Introduction

Since Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS) was introduced by Comisarow and Marshall in 1974, many research groups have gone into mass measurement accuracy (MMA) enhancement using the advantages of high resolving power of FT-ICR MS in wide mass range with external or internal calibration. MMA is crucial for characterization and identification of small molecules such as natural products, drug substances, metabolites as well as peptides. Due to simultaneous detection of calibrants and analytes, internal calibration shows higher MMA by compensating time-dependent tolerances. It has been reported to improve MMA using matrix assisted laser desorption ionization (MALDI) FT-ICR MS.

Method

All mass spectra were obtained using positive ion mode on a MALDI FT-ICR-MS with a 15 T superconducting magnet (ApexQe Bruker Daltonics, Bremen, Germany) . Desorption/ionization was carried out with 355 nm Nd : YAG laser of 6 ns pulse width. Carbon soot (CS ; combustion from liquid butane) was prepared for a source of carbon clusters and used for a calibrant as well as for a matrix of positive ion MALDI FT-ICR MS. The enhancement of MMA by internal calibration with a number of standard peaks from CS was demonstrated via analysis of a peptide mixture, which was directly spotted onto CS layer.

Results

Carbon clusters (soot) with 24 u mass spacing were generated in the range of *m/z* 720-2500 and successfully adopted for internal calibration of a peptide mixture which is usually used as internal standards. With the high resolution carbon cluster peaks, the mass accuracy of internal calibration was improved in 15 T MALDI FT-ICR MS. The average mass error of analytes was ~29 ppb and the RMS error of ~31 ppb.

PMo-020
13:30 – 14:40

Identification of epitopes recognized by monoclonal antibodies raised against the meningococcal factor H binding protein

Agnese Faleri[1,2], Sebastien Brier[2], Laura Santini[2], Silvana Savino[2], Marzia Monica Giuliani[2], Mikkel Nissum[2], Mariagrazia Pizza[2], Vega Masignani[2], Nathalie Norais[2]

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Keywords:
Epitope mapping, H/D exchange

Novel aspects:
Mapping of epitopes recognized by different monoclonal antibodies raised against the fHbp of Neisseria meningitidis using Hydrogen/Deuterium exchange coupled with Mass Spectrometry.

Abstract:
The factor H binding protein (fHbp) is included in the multicomponent 4CMenB developed to prevent disease caused by *Neisseria meningitidis* serogroup B. fHbp is a 27-kDa membrane-anchored lipoprotein that was discovered by reverse vaccinology. It binds to the complement factor H regulator and down-regulates the alternative pathway thus allowing survival of the bacteria in human plasma. fHbp has also the capability to induce bactericidal antibodies in mice and humans, and it was proposed that antibody binding to fHbp could increase the immune response by preventing the bacteria to inhibit the complement alternative cascade.
The identification of protective epitopes is an important step to understand the mechanism of an effective immune response and to improve the rational design of more protective antigens. In this study, we investigated the interactions between fHbp and different monoclonal antibodies using hydrogen-deuterium exchange associated with mass spectrometry (H/DX-MS) . The H/DX-MS approach relies on the differential solvent accessibility of the free and bound antigen to a deuterated buffer. Thus, the antigen epitopes in interaction with the antibodies are protected from the solvent and incorporate less deuterium compared to the free antigen. We report here the mapping of the epitopes recognized by different monoclonal antibodies and present a structural comparison of the different antigenic regions identified.

PMo-021
11:10 – 12:20

Variable Temperature Ion Mobility Mass Spectrometry of Multimeric Protein Complexes

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Keywords:
ion mobility, variable temperature, protein complexes, thermal stability

Novel aspects:
Variable temperature IM-MS studies investigate the influence of buffer gas temperature on the mutlimeric protein structures, and highlight the difference between the gas phase and solution thermal stability.

Abstract:
Despite an increasing success of ion mobility mass spectrometry (IM-MS) to study intact biomolecules, there are still some controversies regarding structure preservation in the gas phase and data calibration obtained from the commercially available travelling wave ion mobility spectrometry (TWIMS) instrument Synapt HDMS. The issue with any mobility analyzer used to investigate the conformations of biomolecules is that the measurement *itself* may alter the conformation of the ion. Application of high injection energies and long ion storage times can cause structure collapse or reorganisation *via* unfolding. Recent studies show, that the ion effective temperature within TWIMS for small proteins can exceed 500 K which will cause an ion to re-order to more elongated conformations under typical settings recommended by the manufacturer. We set out to investigate the influence of buffer gas temperature on the mutlimeric protein structures and gain more insights into the thermal stability those complexes.

Ion mobility and mass spectrometry data of mutlimeric complexes was acquired on a home built linear temperature variable drift tube DT IM-MS instrument. Ion species selected for this study were trimeric Protein X (52 kDa) , and two commercially available tetrameric protein complexes : avidin (64 kDa) and concanvalin A (102.6 kDa) . All three systems yield stable ion currents and have simple preparation requirements. Prior to IM-MS analysis, the sample buffer was exchanged to 100 mM ammonium acetate using chromatography spin columns. The data were obtained at different buffer gas (helium) temperatures ranging from 200 to 500 K to examine the thermal stability of those protein complexes, their collapse, unfolding and complex dissociation.

All three protein complexes under investigation present narrow charge state envelopes (protein X trimer n =13-16, avidin tetramer n = 15-18 and concanvalin A tetramer n = 21-24) . Smaller complexes such as trimeric protein X and avidin) present a shift towards higher charge states upon exposure to higher temperatures such as 400 K. Ion mobility data analysis show a decrease in collision cross sections (CCSs) of those complexes in comparison to CCSs determined at 300 K, suggesting a structural collapse. Moreover, in case of Protein X the stochastic increase in CCSs usually observed with increasing charge state resulting from protein unfolding due to coulombic repulsions, is no longer observable ; the CCSs remain *more-less* constant across charge states, again supporting the complex collapse. When exposed to temperatures above 500 K, the protein complexes dissociate into monomeric (Protein X, avidin, concanvalin A) or dimeric (concanvalin A) species. Capturing the ions prior to the dissociation (at 470 480 K) reveals a drastic increase in the CCSs associated with protein unfolding. Based on the IM-MS data, we speculate that investigated protein complexes exposed to high temperatures collapse first prior to unfolding. Interestingly, cooling down the drift cell to 200 K allows preservation of tetrameric concanvalin A, which otherwise exhibits a significant amount of dimeric and monomeric species even at 300 K. Presented variable temperature IM-MS studies pinpoint the importance of the analysis temperature for protein structural studies, and highlight the difference between the gas phase and solution thermal stability.

PMo-022
13:30 – 14:40

Full H/D Exchange for Improving Small Molecule and Peptide Identification with Electrospray Ionization

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Keywords:
Full H/D exchange, gas-phase H/D exchange, electrospray

Novel aspects:
Complete H/D exchange of small analytes and peptides and their analysis by ESI-MS and -MS/MS without significant loss of label.

Abstract:
N-, O-, and S-bound hydrogens undergo fast H/D exchange in solution. Determination of the number of exchangeable hydrogens in an analyte strongly substantiates the confidence of mass spectrometric identification. So far complete H/D exchange of peptides with subsequent analysis by MS is reported only sporadically [1] . In this study we show the systematic investigation of gas-phase and solution H/D exchange for a variety of compound classes with the aim of full H/D exchange. As a result of this full exchange, MS and MSMS spectra of peptides are better suited for *de novo* sequencing via CBS [2] and for database-linked peptide identification. The identification of unknown analytes by MS is generally put on a more reliable basis using full H/D exchange as additional analytical tool.

By in-solution H/D exchange, virtually complete exchange can be achieved for N-, O-, and S-bound hydrogen atoms in intact molecules (metabolites, amino acids, lipids and peptides) . In an electrospray ion source, H/D exchange may occur by collisions with gas-phase components containing exchangeable hydrogens [3] . While this phenomenon is of disadvantage for exact measurements of the number of exchangeable hydrogens, it offers the possibility for gas phase introduction of deuterium within an ESI source. For small molecules, we observed that using a D 2 O-saturated ESI source, hydrogens present in -COOH, -OH, -SH, -NH2, non-amide -NH, as well as in phosphate and phosphate esters groups efficiently undergo H/D exchange. Amide hydrogen in peptides is only poorly exchanged under these conditions, as confirmed by gas phase in- and out-exchange of deuterium. However, full H/D exchange could be established using highly enriched D 2 O (99.9 % atom% D) as solvent plus a D 2 O-saturated ion source atmosphere. In this way, degrees of deuteration of up to 99.8 atom% at all exchangeable positions could be achieved. Tandem mass spectra of fully deuterated peptides show that b- and y- ions carry the number of deuterium atoms calculated on the basis of their structure. Thus, full H/D exchange is avaluable tool for improving de novo and database-linked peptide sequencing.

PMo-023 SALDI-MS of oligopeptide using gold nanorods on ITO plates.

11:10 – 12:20

Masanori Fujii[1], Naotoshi Nakashima[1,2,3], Yasuro Niidome[1,2]

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Keywords:
Gold nanorods, SALDI-MS

Novel aspects:
We demonstrated a high sensitivity SALDI-MS measurement to the angiotensin on an ITO plate using photoabsorption of the gold nanorods.

Abstract:
Surface Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SALDI-MS) is frequently used for analysis of biomolecules. In modern SALDI-MS measurements, nanostructured surfaces or nanoparticles absorb photons and assist in desorption and ionization of sample molecules. In this work, we detected of oligopeptide (angiotensin I) in SALDI-MS measurements using gold nanorods fixed on an indium-thin oxide (ITO) plate. The gold nanorods on the ITO plate are expected to capture and concentrate the target molecules.

A solution of the prepared gold nanorods with aspect ratios of about 5 (51 ± 7 nm and 9.7 ± 1.1 nm in longitudinal and transverse directions, respectively) was centrifuged twice at 15000 g for 10 min and then decanted to remove excess CTAB molecules from the solution. After this, gold nanorods were coated with poly (sodium styrenesulfonate) (PSS) to obtain poly-anionic surfaces. The PSS layers contributed to decrease the concentration of CTAB in the solution and stabilize the colloidal dispersion of the gold nanorods in the absence of CTAB. An ITO plate modified with 3-aminopropyltriethoxysilane (APTES) was immersed in an aqueous solution of the PSS-coated gold nanorods to deposit the anionic nanorods on the cationic surfaces. The nanorod-ITO plates that were dropped and dried angiotensin I (100 mL) were used for SALDI-MS measurements using a MALDI-MS instrument (Autoflex III, Bruker Daltonics) . The light source was a nitrogen laser oscillated at 337.1 nm. An aqueous solution of trifluoroacetic acid (0.1wt.%, 100 mL) was dropped and dried onto the plate before measurements.

The extinction spectra of the nanorod-ITO plate are shown the two SP bands near 520 and 900 nm. This indicates that the gold nanorods were deposited on the ITO plate. The mass spectra of the nanorod-ITO plate with angiotensin (0.5 mM 10 fM) are shown a peak of protonated angiotensin (m/z = 1297) . Even when adilute angiotensin solution was dropped on a nanorod-ITO plate (10 fM) , a peak of protonated angiotensin was found at m/z = 1297 with a good signal/noise ratio (S/N = 11) . In the absence of gold nanorod, no signal was detected even when 10 mM angiotensin I was dropped on an ITO plate. In the absence of angiotensin I, the nanorod-ITO plates with and without trifluoroacetic acid showed several peaks, but there were no peaks at m/z = 1297. These control experiments indicate that the peaks at m/z = 1289 and 1291 are background signals from the PSS or APTES on the nanorod-ITO plate, but the signal at m/z = 1297 of angiotensin I comes from the SALDI processes induced by the photoabsorption of gold nanorods.

The ITO plate, which was modified with gold nanorods, was found to have effects in collecting angiotensin I molecules close to the gold nanorods, and the SALDI processes that were induced by the photoabsorption of the gold nanorods efficiently contributed the desorption and ionization of the angiotensin I.[1,2)]

References

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2) Y. Nakamura, Y. Tsuru, M. Fujii, Y. Taga, A. Kiya, N. Nakashima and Y. Niidome, *Nanoscale*, **3**, 3793-3798 (2011) .

PMo-024
13:30 – 14:40

Collision/Reaction cell for ICP-MS - a new Concept for an improved removal of low masses

Lothar Rottmann, Jung Gerhard, Tomoko Vincent

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Keywords:
ICP-MS, collision cell, new cell design with stepped or curved rods, interference removal

Novel aspects:
The sensitivity of a collision cell for ICP-MS is significantly improved by using a rod structure with variable cross-section.

Abstract:
One of the fundamental challenges in ICP-Q-MS, polyatomic interferences can be suppressed with the use of a collision/reaction cell. Although a universal gas mixture can be suitable for most routine applications, some analytes in more challenging matrices will either benefit from an alternative gas or more aggressive cell conditions to preferentially suppress or remove the polyatomic interferences. However, in the reaction mode it is also preferable to remove the reacting gas ions and other low mass ions along the cell.

A new design of a collision cell comprising flatapole rods with a curved or stepped shape was developed. This allows a high transmission caused by a larger r_0 at the entrance of the cell and a low mass cut-off at the lower r_0 e.g. in the middle of the cell. The underlying principle is the variation of q along the collision cell. This allows achieving for the same RF amplitude high sensitivity and robustness as well as sufficiently high low-mass cut-off to ensure elimination of reaction products.

Experiments were carried out that clearly showed the following performance improvement :
· High transmission of the system even for low masses in kinetic energy discrimination (KED) mode in a He pressurized cell. At the iCAP Q > 400 cps for ^{7}Li could be detected in a 1 ppb standard solution in this mode.
· Low-mass cut-off in order to remove unwanted masses of lower mass. The background from high intensity peaks of e.g. ^{40}Ar is completely removed at higher masses and thus allows higher signal-to-noise at these masses.
Excellent interference removal properties. In KED mode a value of > 20 for the ratio of the intensity of 1 ppb ^{59}Co and the interference of $^{35}Cl^{16}O$ could be achieved easily for a 1.5 % HCl matrix.

PMo-025
11:10 – 12:20

Applications of online- bioaffinity- mass spectrometry to structure and affinity determination of neurodegenerative proteins from brain material

Stefan Slamnoiu[1], Camelia Vlad[1,2], Adrian Moise[1], Mihaela Stumbaum[1,2], Thomas Gronewold[2], Markus Perpeet[2], Michael Przybylski[1]

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Keywords:
affinty mass spectrometry, SAW-ESI-MS, biosensor, neurodegenerative proteins, alpha synuclein

Novel aspects:
The new affinity-MS method based on the online coupling of SAW-biosensor to ESI-MS provides information of biopolymer interactions at molecular level, enabling affinity detection and quantification, and ligand structure identification.

Abstract:
Introduction:
In the analysis of affinity interactions biosensors are a key analytical tool, being able to detect and quantify bioaffinity bindings. Nonetheless, biosensors do not provide the chemical structure identification of ligands, for which mass spectrometry is a powerful tool [1]. Here we show that the online combination biosensor- mass spectrometry provides simultaneous chemical structure identification and quantification of bioaffinity interactions.

Methods:
In this study we report affinity interaction and structural studies of antigen-antibody and peptide-protein complexes, as well as of biological samples : mouse brain homogenate containing α-Synuclein and human CSF containing Aß peptides. The SAW-biosensor was directly coupled to (i) ESI ion trap, and (ii) ESI FT-ICR-MS. The analysed complexes included Aß (1-16) peptide / anti- Aß (1-16) antibody ; Aß (12-40) / anti- Aß (17-28) antibody ; Tau protein (isoform 2N/4R) / anti-Tauspecific antibody (clone TAU5) ; melitin / calmodulin, and α-Synuclein / anti- α-Synuclein antibodies. An anti- α-Synuclein antibody was used for the affinity characterisation of mouse brain homogenate, while anti- Aß antibodies were employed in the analysis of human CSF. Antibodies / proteins were immobilised via a thiol linker on the surface of a gold chip inside the biosensor, and their interactions with the specific peptide or protein were monitored with the SAW biosensor. Using the online interface developed in our laboratory, the bound peptide or protein was eluted at acidic conditions from the gold chip and analysed by ESI mass spectrometry, which provided the structural characterisation of the ligands [1].

Results:
The online combination of SAW biosensor and mass spectrometry allowed the structural identification and affinity determination of affinity-bound Tau, Aß and α-Synuclein. The mass spectrometric characterisation provided details on post translational structure modifications of proteins and peptides present in biological samples, while the analysis of the binding kinetics delivered the K_D values for the antibody-antigen interactions. The key achievement of the method consists in the ability to structurally characterize specific biopolymer molecules (Aß, α-Synuclein) , occurring at low concentrations in biological material. Using this new hybrid tool, screening for biomarkers of neurodegenerative diseases will become more efficient and accurate, leading to easily quantifiable and interpretable results.
(Supported by grant KF2026662 from the Bundesministerium für Wirtschaft und Technologie, Berlin Germany)

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PMo-027
11:10 – 12:20

Determination of Opiates in Dried Blood Spots using Novel Flow-through Technology coupled to LC/MS/MS

Kenneth Lewis[2], Dennis NAGTALON[1]

[1]Agilent Technologies, [2]OpAns

Keywords:
Dried Blood Spot, LC/MS, Opiates, Blood

Novel aspects:
Automated flow-through dried blood spot LC/MS analysis for opiates in blood

Abstract:
Dried blood spot technology (DBS) coupled to highly sensitive LC/MS/MS systems promises significant advantages in bioanalytics both in the toxicology and pharmaceutical industry. However, two major disadvantages that limit the acceptance of DBS technology are : (1) the laborious, time-consuming, error prone sample pre-treatment required, and (2) poor assay sensitivity due to reduced sample volume. An automated card extraction system virtually eliminates off-line sample preparation with direct online sample extraction of DBS cards without the need for hole punching while triple quadrupole mass spectrometry technology provides unparalleled sensitivity and superior quantitation for the low sample volumes typically encountered with DBS analyses. This presentation will describe our application of this integrated analysis system to the quantification of opiates in blood in the ng/mL concentration range.

PMo-029
11:10 – 12:20

Optimization of serum N-linked glycan profiling using MALDI-TOF mass spectrometry

Yongha In[1,2], Heysun Maeng[1,2], Sun Young Ahn[1,2], Chang Won Park[1,2], Yangsun Kim[1,2]

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Keywords:
glycan, glycomics, homogeneous, crystallization, reproducibility

Novel aspects:
Uniform and homogeneous crystallization of matrix material by automatic matrix dispensing system is of critical importance to consistent and quantitative analysis of glycans with MALDI-TOF MS

Abstract:
Most of currently available cancer biomarkers are glycoproteins such as CA125 (MUC16), alpha-fetoprotein (AFP), and CA 15-3 (MUC 1), and many tumor antigens are derived from glycans which are supposedly shed by glycoproteins. In addition, quite a few individual proteins have been reported to change their glycosylation patterns in cancer. On the basis of these findings, the study of global pattern of protein glycosylation, so called 'glycomics', is now considered to be highly promising source of new cancer biomarkers. The method of choice for the precise profiling of glycans is mass spectrometry (MS), which provides a wide range of analytical power to successfully distinguish molecules with tiny differences in the molecular mass. A couple of successful applications of glycan analysis by MS to the identification of potential biomarkers have been already published. Here, we report the optimization of high-throughput profiling of N-linked glycans by MALDI-TOF MS with high reproducibility. N-linked glycans were released from glycoproteins by peptide N glycosidase F (PNGase F), and analyzed by MALDI-TOF MS. We focused on the minimization of variations in MS peak intensities that could be generated in each steps of MALDI-TOF MS. For the stable consistency of the analysis, uniform and homogeneous crystallization of the matrix material on the sample spot was found to be critical. Therefore, we developed automatic matrix dispensing system so as to enhance the homogeneous matrix preparation, and tested its performance regarding reduced variations in the MS data. Through the correlation of MALDI parameters, optimized MALDI analysis conditions were determined, leading to stable reproducibility. The coefficient of variations (CV) in normalized intensities of glycans decreased below 5 %, and the variations in absolute intensity were in a range of 0.69-9.25% for 32 samples, indicating significant improvement in minimizing variations of MS data. These results suggest that uniform and homogeneous crystallization of matrix material by automatic matrix dispensing system is of critical importance to consistent and quantitative analysis of glycans with MALDI-TOF MS.

PMo-030
13:30 – 14:40

Analysis of Panax ginseng extracts by comprehensive Two-Dimensional Ultra High Performance Liquid Chromatography coupled with IT-TOF

Tatsunari Yoshida[1], Shin-ichi Yaguchi[2], Tsutomu Nishine[1], Hirohisa Mikami[1]

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Keywords:
comprehensive 2D-LC, LC × LC, two-dimensional-UHPLC, LCMS-IT-TOF, Panax ginseng extracts

Novel aspects:
In this study, we employed comprehensive two-dimensional UHPLC coupled with LCMS-IT-TOF for the analysis of Panax ginseng extracts, a popular Chinese herbal medicine.

Abstract:
The great sample complexity often overgrows the separation power of mono-dimensional (1D-) HPLC techniques ; in such cases implementation of multidimensional chromatographic systems is mandatory. In this respect, comprehensive two-dimensional liquid chromatography (2D-LC) is a powerful tool for the analysis of complex samples including pharmaceutical, biological, and natural products. The favourable features of 2D-LC were already demonstrated more than thirty years ago ; since then, a lot of efforts have been put in the development of multidimensional techniques of ever higher peak capacity. Recently, UHPLC (Ultra High Performance Liquid Chromatography) has been successfully employed to remarkably decrease analysis time in the second dimension of comprehensive LC system, and it is becoming popular to speed up 2D-UHPLC separations.

In this study, we employed two-dimensional UHPLC for the analysis of Panax ginseng components, a popular Chinese herbal medicine. A combination of RPLC (low pH) x RPLC (high pH) was selected and the chromatographic behavior of five major ginsenosides (ginsenoside Rg1, Re, Rb1, Rc, and Rd) and other minor compounds was monitored. All experiments have been run on a 2D-UHPLC system (Shimadzu Prominence) , and interfaced through ESI to an hybrid mass spectrometer (Shimadzu LCMS-IT-TOF) , which possesses both MSn ability of an ion trap and the excellent resolution and mass accuracy of time-of-flight. The LCMS-IT-TOF and Composition Formula Predictor Software allowed the structural assignment of ginsenosides in Panax ginseng with similar formula below 5 ppm for analysis.

PMo-031
11:10 – 12:20

Influence of Sample Preparation Techniques on the Sensitive Detection of Peptides by MALDI-MS

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Keywords:
MALDI preparation technique, highly sensitive analysis

Novel aspects:
The optimization of matrix concentration and solvent system significantly improved the quality of MALDI mass spectra of peptides.

Abstract:

Introduction :

The quality of MALDI mass spectra of peptides is known to be significantly influenced by sample/matrix preparation procedures. So far, many different kinds of sample/matrix preparation methods have been developed. The most widely used among them is a dried-droplet method with the use of a saturated matrix solution on a stainless steel sample plate. Systematical understanding, however, of preparation techniques in terms of highly sensitive detection of low-abundance peptides without using special plate or anything complicated have been lacking in detail. To this end, we started a systematic study on optimizing conditions for matrix concentration and solvent system for both sample and matrix. Results demonstrated a detection limit of below 10 amol peptides using alpha-cyano- 4 -hydroxycinnamic acid (CHCA) as a matrix with sufficient signal intensities.

Methods :

A 0.5 uL volume of peptide solutions with an equal volume (0.5 uL) of matrix solutions were mixed on a stainless steel MALDI plate (Shimadzu/Kratos, UK) and allowed to dry at ambient atmosphere. Sample and matrix solutions were prepared using 0.1% (v/v) aqueous TFA for acidification. MALDI-TOF MS (*AXIMA Performance*™ ; Shimadzu/Kratos, UK) measurement using CHCA as a matrix was performed in positive reflectron mode. MALDI-QIT-TOF MS (*AXIMA Resonance*™ ; Shimadzu/Kratos, UK) measurement using 2,5-dihydroxybenzoic acid (DHB) as a matrix was performed in positive mode. Peptides and protein digest standards were purchased from Waters (USA) .

Results :

To optimize the concentration of CHCA in matrix solutions, we examined several points of the concentrations ranging from 0.01 to 10 mg/mL. The CHCA concentration dependence of MALDI-MS response was examined by using the signal-to-noise ratio. Results demonstrated the extremely high S/N ratio of peptide peaks in MALDI mass spectra at a CHCA concentration of around 0.1 mg/mL. The enhanced MALDI-MS response (about 10-100 times) compared with the use of a saturated (10 mg/mL) matrix solution was observed. For example, limits for the detection of human ACTH 18-39 was 10 amol under optimal condition using the dried-droplet technique in MALDI-TOF MS measurement (positive reflectron mode) . Furthermore, the effect of varying solvent systems for sample and matrix preparation was examined in terms of matrix solubility, reducing adsorption of sample to plastic tubes, and small deposition on target plate. The condition by optimizing matrix concentration and solvent system obtained in this study using ACTH 18-39 was verified with four other peptides and achieved significant improvement of sensitivity by two to three orders of magnitude, comparing with the conventional conditions. The optimization of concentration and solvent composition of both CHCA and DHB matrices significantly improved the quality of MALDI mass spectra of peptides, and details will be discussed in this session.

PMo-032
13:30 – 14:40

Development of modified micro-array MALDI plates with carboxylated dopamine for the analysis of small molecules.

Duhee Park[1,2], Hyunjung Seo[1,2], Chang Won Park[1,2], Yangsun Kim[1,2]

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Keywords:
dopamine, matrix, carboxylation, coating, DHB

Novel aspects:
Micro-array carboxylated dopamine plates can efficiently assist the ionization of samples for the detection of small molecules without matrix

Abstract:
Dopamine in mussel is known as adhesive material, which is abundant in the proteins found at the interface between adhesive pads and opposing surfaces, and helps mussels bind to organic or inorganic surfaces. This adhesive characteristics and molecular structure of dopamine is similar to 2,5-dihydroxybenzoic acid (DHB) with substitution of a carboxyl group to an amine group. Since DHB is one of the preferred matrix material for MALDI-TOF mass spectrometry (MS) , we attempted to make dopamine-coated disposable target plates for the analysis of small molecules by MALDI-TOF MS, and tested the plates for possible use as a sample platform for MALDI-TOF MS without additional matrix material, anticipating that dopamine on the plate can play a role of matrix material. Micro-patterning with chemical etching and photolithography were used to make an array of 50-500 μm hydrophilic spots with 100-300 μm gap on hydrophobic plates. It has 5000 more spots in comparison with those of standard microscope slide. Patterned stainless steel surfaces have been coated with 3,4-dihydroxy-L-phenylalanine (DOPA) by immersing the stainless steel plates in DOPA solution with gentle shaking. The adhesion of dopamine is confined only within the hydrophilic spot area. The thickness of coated film increased with coating time, and the color of hydrophilic spot area turned dark brown when it was monitored by a microscope. A homogeneous and fine surface structure was shaped eventually. The ionization of some small molecules on the dopamine-coated plates for MALDI process of MS was increased when observed in MS spectra of the small molecules, indicating that coated dopamine layer on the sample spot could replace MALDI matrix material. Further modification (carboxylation) of dopamine-coated surface with succinic anhydride has been performed and applied to the detection of small molecules by Laser Desorption Ionization MS. The molecular ion peaks were obtained from carboxylated dopamine plates and compared with those from different plates. The ionization yields from micro-array carboxylated dopamine-coated plates were comparable with DHB matrix spots. The clear identification of small molecules by MALDI-TOF MS was possible due to the absence of interference from matrix peaks. In conclusion, micro-array carboxylated dopamine plates can efficiently assist the ionization of samples for the detection of small molecules without matrix, and can provide a preferred sample platform for high throughput MALDI-TOF MS analysis of small molecules.

PMo-033
11:10 – 12:20

Bonded Zwitterionic Stationary Phases and Mass Spectrometric Detection for Bioanalysis

Patrik Appelblad, Wen Jiang, Jonsson Tobias, Petrus Hemstrom

Merck SeQuant, Umea, Sweden

Keywords:
LC/MS, HILIC, Liquid chromatography, Detection sensitivity

Novel aspects:
Hydrophilic interaction chromatography (HILIC) has excellent compatibility and provides enhanced detection sensitivity when combined with mass spectrometric (MS) detection.

Abstract:
Introduction

Hydrophilic interaction chromatography (HILIC) is a useful tool for separation of polar compounds in biofluids.HILIC has excellent compatibility and provides enhanced detection sensitivity when combined with mass spectrometric (MS) detection. HILIC uses a high percentage of organic solvent in the mobile phase, hence easy to volatilize in the detector interface.

Biofluids is generally of aqueous character and contain many polar compounds, and their metabolites. The liquid part of blood, devoid of cells and platelets, is termed either plasma or serum depending on how the sample has been prepared. By applying protein precipitation protocols prior to analysis, matrix (proteins, salts, minerals and lipids) can effectively be removed and allow sensitive methods to be developed. In practice, this means adding a volume portion of plasma, serum, or urine in a minimum of four volume portions of organic solvent to stimulate protein precipitation. Besides effective matrix removal, the procedure makes the biological sample perfectly aligned to a HILIC separation, and large sample volumes can be injected without a risk of compromising the separation efficiency. This poster will illustrate how different bonded zwitterionic HILIC phases have been utilized for bioanalytical purposes.

PMo-034
13:30 – 14:40

Quantitative Analysis of Free and Total Thyroid Hormones in Serum With and Without Online Sample Cleanup using LC/MS/MS

Rory Doyle, Kevin McCann

AGILENT TECHNOLOGIES

Keywords:
thyroid, hormones, LC/MS, serum, online sample cleanup

Novel aspects:
Analysis of thyroid hormones in serum by LC/MS/MS with/out online sample cleanup

Abstract:
Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) has become an essential tool for small molecule quantitation due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis of multiple analytes. Thyroid hormones can be challenging compounds to analyze due to the low levels relevant to clinical research. In order to address this challenge, sensitive liquid chromatography-tandem mass spectrometry methods for the simultaneous analysis of Thyroxine (T4),3,3',5-Triiodothyronine (T3) and 3,3',5'-Triiodothyronine (rT3) in serum samples were developed.

Both positive and negative ionization techniques were investigated, but optimal results were achieved using positive electrospray ionization (ESI). Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard. Final concentrations were calculated by comparing the response of the analyte to a known concentration of internal standard and plotting the result on a calibration curve developed using stripped human serum spiked with standards. Standard liquid chromatography (LC) was used initially.The LC system was also configured for sample enrichment, comprised of two pumps, a 2 position/6 port valve, an enrichment column and a reverse-phase C18 analytical column to assess the added value of online sample cleanup. Methods were compared using measurements made in serum Standard Reference material (SRM9971). The LC-MS/MS parameters were optimized and calibrated over the range of 1 pg/ml to 500 pg/ml for free and 1 ng/ml to 500 ng/ml for total Thyroid hormone concentrations. The LOQ was the lowest calibrator. The calibration curves show excellent linearity and reproducibility across the entire range of analysis.

PMo-035
11:10 – 12:20

Selective introduction of 4-amidinobenzyl moiety into peptide N-terminus for straightforward de novo sequencing

Toru Yoshikawa, Masahiro Miyashita, Hisashi Miyagawa

Kyoto University, Kyoto, Japan

Keywords:
de novo sequencing, charge derivatization, fragmentation, selective modification, peptide

Novel aspects:
This study offers much easier and more straightforward sequence determination of peptides from complex mixtures using chemical modification approach

Abstract:
MS/MS has become a major technique for protein and peptide sequencing, owing to its high sensitivity, rapid analysis, and applicability to complex peptide mixtures. In particular, *de novo* peptide sequencing (that is, elucidation of amino acid sequence without the prior knowledge of the sequence information) is powerful when the target peptides are from unexplored organisms lacking genomic/proteomic information, or those undergone post-translational modifications. However, due to incomplete fragmentation of the peptide backbone, *de novo* peptide sequencing remains a challenging task. In this context, we have demonstrated that N-terminal derivatization with a 4-amidinobenzoic acid greatly improves peptide fragmentation under low-energy CID conditions, such as in a triple quadrupole or an ion trap mass spectrometer. 4-Amidinobenzoyl moiety can be introduced into N-terminal amino groups by using a succinimide active ester method, which is commonly used for derivatization of primary amines. However, introduction of 4-amidinobenzoyl moiety usually occurs not only at N-terminal a-amino groups, but also unfavorably at side chains of lysine and tyrosine residues by this method. This often results in the production of a complex mixture which makes the analysis even more difficult. Recently, a new method for selective modification at N-terminal a-amino groups of peptides using an oxidative amide synthesis method via metalloporphyrin-catalyzed aromatic alkyne oxidation was reported. In this study, we propose a new method to selectively introduce 4-amidinobenzoyl moiety to the peptide N-terminus by utilizing this reaction.

As an alkyne carrying a 4-amidinobenzoyl moiety, *N*-(4-ethynylphenyl)-4-amidinobenzamide was designed, and synthesized by mixing 4-amidinobenzoic acid and 4-ethynylaniline in the presence of BOP reagent in dry pyridine. The product was purified by HPLC. Metalloporphyrins [Mn(2,6-Cl_2TPP)Cl] were prepared by refluxing porphyrins (2,6-Cl_2TPP) and $MnCl_2$ in DMF. Peptide derivatization with *N*-(4-ethynylphenyl)-4-amidinobenzamide was accomplished as follows : to a solution of lysine-containing peptides in 50 % aqueous acetonitrile, *N*-(4-ethynylphenyl)-4-amidinobenzamide (500 eq), Mn(2,6-Cl_2TPP)Cl (6 eq) in a $NaHCO_3$ aqueous solution containing H_2O_2 were added. The mixture was kept at room temperature overnight, and partially purified with a MonoSpin C18 spin column (GL Sciences, Inc) to remove the unreacted reagents before LC/MS^n analysis. The LC/MS^n analysis was performed using Shimadzu LCMS-IT-TOF hybrid mass spectrometer. All mass spectra were obtained in a positive mode.

LC/MS analysis of the reaction solution showed that only a single moiety of *N*-(4-ethynylphenyl)-4-amidinobenzamide was introduced into each of the tested lysine-containing peptides in a high yield without significant production of by-products. Product ion spectra of derivatized peptides confirmed that the N-terminal a-amino group was specifically modified and that the lysine and tyrosine side chains remained intact. The improving effect of amidino benzoyl moiety on the peptide fragmentation was similar to that observed in the case of derivatization with 4-amidinobenzoic acid using the succinimide active ester method. In conclusion, we have established a method that can selectively derivatize the N-terminal a-amino group for *de novo* peptide sequencing. This method offers much easier and more straightforward sequence determination of peptides from complex mixtures.

PMo-036
13:30 – 14:40

Extractive electrospray ionization mass spectrometry (EESI-MS) for sensitive detection of iodine-129

Zhongchen Wu[1], Yafei Zhou[2], Bin Jia[2], Huanwen Chen[2]

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Keywords:
EESI-MS, iodine-129

Novel aspects:
an EESI-MS based method has been developed for sensitive detection of ultratrace iodine-129 in various liquid samples.

Abstract:
Detection of radioactive species plays an important role in public safety. Due to the high afnity to the human body and very long physical half-life (15.7 million years) , Iodine-129 is considered one of the most hazardous substances and must be monitored with high accuracy and precision.
EESI is an ambient ionization technique targeting to direct ionize samples for mass spectrometric detection with minimal (or without) sample pretreatments. Ultratrace uranyl species[1] and its isotopic ratio ($^{235}U/^{238}U$)[2] have been accurately detected using multiple-stage EESI mass spectrometry in natural water samples.
In this work, an EESI-MS based method has been developed for sensitive detection of ultratrace iodine-129 in various liquid samples. The base peak at m/z 129 ($^{129}I^-$) was adopted for quantification. The calibration curve showed good linearity within a wide range of 6.5-208 ppt (R=0.996) . The limited detection (LOD) is 1.38 ppt, which was measured at three times of the standard deviation of the blank determination, and with the corresponding relative standard deviation (RSD) values of 4.4 %-6.8%.
Since most iodine species are easy to be enriched and absorbed in liquids in the form of ions, our method offers a rapid, sensitive and specific approach for the detection of iodine-129 ions, providing potential applications many cases including nuclear leakage or nuclear explosions.

Acknowledgement(s)
Financial support from Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation (JXMS201106) is highly appreciated.

References
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2. Chunxiao Liu, Bin Hu, Jianbo Shi, jianqiang Li, Xinglei Zhang and Huanwen Chen. Determination of uranium isotopic ratio (235U/238U) using extractive electrospray ionization tandem mass spectrometry. J. Anal. At. Spectrom., 2011,26, 2045-2051

PMo-037
11:10 – 12:20

De novo peptide sequencing facilitated by N-terminal charge derivatization with high proton affinity

Yoichiro Nihashi, Hiroyuki Awane, Masahiro Miyashita, Hisashi Miyagawa

Kyoto University, Kyoto, Japan

Keywords:
de novo sequencing, peptide, derivatization, stable isotope-labeling, low-energy CID

Novel aspects:
The approach utilizing derivatization reagents with high proton affinity as shown in this study offers a novel de novo peptide sequencing method under low-energy CID conditions.

Abstract:
Recent advances in various tandem mass spectrometric techniques have enabled the facile determination of the amino acid sequences of peptides. In addition, the development of comprehensive databases of protein primary sequences predicted from the genomic sequences of various organisms has boosted the efficiency with which proteins can be identified by mass spectrometry. However, since database coverage is limited to the components of major organisms in most cases. Amino acid sequencing without the assistance of a database (de novo sequencing) is still essential in the identification of peptides and proteins from unexplored organisms.

De novo sequencing can be carried out by interpreting mass differences between a series of consecutive ions of N- or C-terminal fragments generated by collision-induced dissociation (CID) . However, fragmentation of peptides often gives complicated and incomplete product ion spectra, making de novo sequencing difficult. Newly developed mass spectrometric techniques for better fragmentation, such as ECD or ETD, and for highly accurate mass measurements, such as FT-ICR mass spectrometry or Orbitrap, have greatly improved the efficiency of de novo sequencing ; however, there still remain difficulties in obtaining mass spectra that allow easy identification of key fragment ions for sequencing. The difficulties come mainly from incomplete fragmentation of the peptide backbone. To overcome these problems, chemical modifications at the N-terminus of peptides have been attempted for more regular and informative fragmentation. The introduction of a positively charged group, such as quaternary or tertiary ammonium and quaternary phosphonium groups, at the N-terminus is known to enhance the relative intensity of N-terminal fragment ions (b-ions) in product ion spectra, significantly facilitating the formation of whole series of b-ions. Similarly, it has been reported that the introduction of a negatively charged group at the N-terminus enhances the generation of C-terminal fragment ions (y-ions) with suppression of the occurrence of b-ions.

In this study, we evaluated the improving effect of N-terminal charge derivatization on peptide fragmentation under low-energy CID conditions using compounds with high-proton affinity. Derivatization reagents containing guanidino or amidino moiety were introduced into the N-terminus of peptides, and their effects on fragmentation were compared using an ion trap mass spectrometer. Among four reagents differing in proton affinity, derivatization with 4-amidinobenzoic acid, which has moderately high proton affinity, brought about the most effective fragmentation in all peptides used in this study. This result clearly indicated that there was an optimal affinity for efficient fragmentation of peptides.

However, even with the aid of derivatization, identification of b-ions was still not very easy in some cases due to the overlap of b-ions with other series of ions, This was especially the case when multiply charged ions were selected as a precursor. In this study, to specifically identify b-ions from other fragment ions, stable isotope-labeling of derivatization reagents was further investigated. 15N was introduced into 4-amidinobenzoic acid, and its effect on specific identification of b-ions was evaluated. As expected, b-ions were effectively identified by comparing the spectra between peptides derivatized with 15N-labeled or non-labeled 4-amidinobenzoic acid, which allowed straightforward interpretations of spectra.

In conclusion, N-terminal derivatization with 4-amidinobenzoic acid undoubtedly improves the peptide fragmentation pattern, which may offer a novel de novo peptide sequencing method under low-energy CID conditions.

PMo-038
13:30 – 14:40

Efficient and rapid multienzymatic limited digestion (MELD) method for complete protein characterization and bottom-up de novo sequencing

Gabriel Mazzucchelli[1], Tyler A Zimmerman[1], Marie-Alice Meuwis[1], Nicolas Smargiasso[1], Michel Degueldre[1], Dominique Baiwir[1], Laurent Leclerq[2], Edwin De Pauw[1]

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Keywords:
de novo sequencing, maximal sequence coverage, multienzymatic digestion, post translational modification, protein characterization

Novel aspects:
By allowing missed proteolytic cleavages, multienzymatic limited digestion method provides better sequence coverage, accurate PTM characterization and allows de novo sequencing of long sequence tag using our “sequence assembly ” software

Abstract:
This method relies on a combination of multienzymatic and limited proteolytic digestions on a purified protein, for complete sequence and posttranslational modification (PTM) characterization. It generates numerous different peptides with miss-cleavages, greatly increasing the probability of analyzing the entire protein sequence.
This 2 hours digestion protocol was validated on several commercial proteins and applied to the identification and localization of Beta-Lactoglobulin posttranslational modifications induced by a reactive metabolite of acetaminophen (NAPQI) . This experiment shows 100 % sequence coverage involving information on peptide identity and modifications localization. Strong identification confidence was obtained due to multiple overlapping peptides matches with the given sequence tag and to a high number of overlapping peptides assignments. A program to assemble amino acid sequences of entire proteins from such data was developed.

HSA, Myoglobin, Lysozyme, Invertase and α-Amylase (5 μg) after reduction-alkylation, were independently digested by proteases mixtures, 2 h, in standard buffer and 2 enzymes mixtures were tested, consisting of Trypsin, GluC and Chymotrypsin at weight enzyme/weight total protein ratios of respectively : 1 /500, 1 /375, 1 /250 for Mix 1 and 1 /3000, 1 /2250, 1 /1500 for Mix 2. Each digest (1 μg) was analyzed by nano-LC ion trap MS (Ultimate 3000, Dionex, Amazon, Bruker) by nano-UPLC Q-TOF (nano Acquity Synapt G2, Waters) with identical 60min. gradient. Data were treated with Data Analysis 4.0, Protein Scape 3.0 (Bruker) and PLGS 2.5 (Waters) . β-Lactoglobulin modifications (NAPQI addition) were induced by activated acetaminophen using an electrochemical flow-through cell (model 5125, ESA Biosciences) .

Complete multi-enzymatic digestion presents the advantage of compatibility with most existing protein sequences, due to high number of accessible specific cleavage sites (6 using trypsin, Glu-C and chymotrypsin) , but the limitation of such a protocol is that the very small proteolytic peptides generated are not compatible for LC-MS analysis. By controlling and limiting digestion reaction time, miss-cleavages are generated and after database searches, the number of unique peptides matching the protein sequence highly increases. The analysis of the five proteins gave the following results : HSA, Myoglobin and Lysozyme were identified with 100% sequence coverage with respectively 421,108, and 49 unique peptides ; Invertase : 75.9% sequence coverage - 158 unique peptides, α-Amylase : 78.5 % sequence coverage - 60 unique peptides. The missing sequence coverages in the last two proteins mainly correspond to sequences known to contain post-translational modifications (PTM) sites. For example, when invertase was *N*-deglycosylated the sequence coverage reached 92.9 % showing clearly which peptides were modified. A comparison analysis of NAPQI modified and unmodified β-Lactoglobulin clearly indicated which cysteine residues were modified by NAPQI. By integrating the extracted ion chromatogram of the unmodified peptides found in common for both conditions, quantification could be achieved and the occupation frequency of this specific modification was evaluated. With our method, at least an average of 10 different peptides covering each sequence stretch of the protein could be obtained. This greatly increased the robustness and the significance of the identifications and the localization of the modifications. The protocol as presented here and tested on several pure proteins digested in solution certainly improves the general “bottom-up “ strategy applied for highly confident protein identification and would allow better protein characterization, even for those having PTMs, such as *N*-glycosylations. Moreover this simple and rapid method could also find helpful applications to assemble amino acid sequence of novel proteins using our “sequence assembly “ software.

PMo-039
11:10 – 12:20

ANALYSIS OF 8 KINDS OF ESTROGENS IN ENVIRONMENTAL WATER BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPH HYBRID TRIPLE QUADRUPOLE MASS SPECTROMRTER

hongyuan Hao, Jinting Yao, Luying Zhou, Hengtao Deng, Qiang Li

Shimadzu (China)Co., LTD. shanghai, china

Keywords:
UHPLC-MS/MS ; Estrogen ; environmental water ;

Novel aspects:
This poster is development of an Ultra fast analysis method for determingestrogens in envirmental water.

Abstract:
A UHPLC-MS/MS method was developed for the determination of Estrogens in environmental water. 8 kinds of Estrogens (Estriol, 17-alpha-Estradiol, 17a-Ethinylestradion, Estrone, 17-beta-Estradiol, Diethylstilbesterol ,hexestrol, dienestrol) were seperated by UHPLC system with a gradient elution program, and detected by Shiamdzu Triple Quadrupole MS LCMS-8030. The linear range was from 0.5 to 500 μg/L with correlation coefficients (r) more than 0.999. Retention times and peak areas results were highly reproducibility. The limit of quantification (LOQ) were less than 2 ng/L for all of Estrogens. This method is rapid, efficient and highly sensitive for quantitative analysis of 8 Estrogens in environmental water.

PMo-040
13:30 – 14:40

Identification of metabolic pathways related to xylose fermentation performance in recombinant yeast by metabolome analysis

Hitoshi MITSUNAGA[1], Danang Waluyo[1], Haruyo Hatanaka[2], Takeshi Bamba[1], Eiichiro Fukusaki[1]

[1]Osaka University, Osaka, Japan, [2]Suntory Business Expert Limited.

Keywords:
xylose, ethanol, performance, PLS, methionine

Novel aspects:
Based on metabolome analysis, PLS might semi-rationally be able to determine the pathway related with xylose fermentation performance.

Abstract:
Xylose is the second most abundant sugar after glucose in the hydrolyzate of lignocellulose. Thus, construction of recombinant yeast that efficiently converts xylose into ethanol became an important task for successful ethanol production from lignocelluloses. However, due to the slow consumption of xylose and relatively high yields of by-products such as xylitol and glycerol, the ethanol yield is very low, which suggests that some metabolic regulations might (have) been induced during xloseassimilation in the recombinant yeast. However, there is no effective strategy of molecular breeding to rationally improve the artificial xylose metabolism, because metabolism in the recombinant yeast has not yet been elucidated. This study aims to develop a semi-rational metabolome analysis-based method for identification of metabolic pathways related to xylose fermentation performance in recombinant yeast.

Seven independent xylose assimilating yeast strains were subjected to the ethanol fermentation experiment in artificial synthetic media containing xylose as a sole carbon source in order to quantitatively evaluate the performance of fermentation. Time course of several important compounds, including xylose, ethanol, glycerol and xylitol were monitored by means of HPLC. Seventy-five and forty-two compounds (117compounds in total) from yeast cells were determined by GC-MS and CE-MS, respectively. Orthogonal projection to latent structure was conducted to reveal the relationship between the 117 metabolites and ten indices of fermentation performance. Analysis of important metabolites for prediction model construction revealed that several amino acids biosynthesis are important for xylose fermentation performance of the recombinant yeast. Several genes which might correlate with these important pathways were suggested to be the target for genetic modification for improvement of xylose fermentation performance. Based on the OPLS analyses, some genetic modification were introduced to the xylose assimilating recombinant yeast. The result of the fermentation test of this recombinant yeast revealed that the modulation of methionine biosynthesis-related genes would contribute to reduction of xylitol production without significant loss of function for both xylose consumption and ethanol production.

To prove the universality of the contribution of methionine for reduction of xylitol production in the recombinant yeast, wild-type strain was subjected to the fermentation experiment with a methionine-rich medium. The result showed that xylitol production was decreased without significant changes in xylose consumption and ethanol production. This suggested that methionine biosynthesis might be generally related to xylitol production in recombinant yeasts.

PMo-041
11:10 – 12:20

Study using synthetic organic acids in Thermo Finnigan LTQ Orbitrap APCI. Application to the acidic fraction, from Brazilian marine oil.

Luzia Koike[1], Eugenio V Santos Neto[2], Rosane A Fontes[2], Rosane N Castro[3]

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Keywords:
Biodegradation, Brazilian oils, Aciod Components

Novel aspects:
- Direct injection in High resolution UPLC-LTQ-Orbitrap APCI (-) ,geochemistry parameters from acid fractions compare whit hydrocarbons and obtain biodegradations parameters

Abstract:
Study using synthetic organic acids as standards in Thermo Finnigan LTQ Orbitrap APCI. Application to the acidic fraction, from Brazilian marine oil.

Autores : Luzia Koike[1]; Rosane Alves Fontes[2]; Eugenio Vaz dos Santos Neto[2]; Rosane Nora Castro[3]; Flávia Airoldi Aguayo [1],Nayara S.Inoue[1], Luiz G.Bonato[1] and Luiz Fernando Lima[1]
1 Campinas State University, Organic geochemistry Group, [2]Petrobras R&D Center,Rio de Janeiro, RJ.and [3] Federal University of Rio de Janeiro, RJ.

Study of biodegradation of the evaporitic marine oils from northeastern Brazil Potiguar Basin named ME-a to Me-d, the acid fractions, were analyzed using Thermo-Finnigan-LTQ-Orbitrap in APCI (-) mode. Standards acids were synthesize : 5a (H) homopregnanic **1** and 5b (H) cholanic **2**with similar basic structures of the steranes (K. Peters and M. Moldowan, 1993 and Raphael Ikan,1993) , 8,13-tetrahydrocopalic **3** and 15-isocopalic acid **4** with similar basic structures to studied terpanos (K. Peters e M. Moldowan,1993) .The compounds were analyzed using isopropanol and toluene (1 : 1) in the APCI source negative mode to study the profiles and fragmentation patterns. Although the main fragments in the all standard spectra show the loss H_2O or CO (William J. Griffths 2003) from the molecular ion, we also see important fragments that are at 217.16 and 191,08 and 249.25 Da, gives respectively, as in steranes and terpanes. In previous work (L.Koike et al., 1992 ; Sidney G. De Lima et al. 2010) the acid fractions originating from Brasilian oils were studied using biodegradation and we derivatized through four steps of the chemical reactions. After this process the yield of the acidic fractions were near 20 % . In the present work the acid fractions were extracted and cleaned up using SPE-NH_2 , increasing the recuperation to 80-90 %. After the extraction and clean up, the samples were injected directly in the LTQ Orbitrap UPLC by APCI in the negative mode. Spectra were acquired at high resolution.
Comparing biomarker parameters from the hydrocarbon fraction with those of the acid fraction reveals that biodegradation increases from ME-a to ME-d. All the related parameters are closely similar in maturity from the ME-a to Me-d and the ratio between steranes/terpanes are characteristic of marine oils. Carboxylic acids can help to refine the classification of degrees of biodegradation of oils, and can be applied to other studies such as secondary cracking of oil, which is a crucial step of quantification. After obtaining sample profiles we used the software COMPOSER^R to summarize the data and related parameters for the acid fraction. Because the software summarizes using the insaturation índex DBE (Double Bond Equivalent) with this software it was possible to see the serial linear acid (DBE 1.5) biodegradations increasing from ME-a to ME-d. In the O_2 (DBE 2.5) we saw linear substituted in monocyclic acids. From the intracelular layer of alicyclic bacyllus, showing increases from Me-a to ME-d. The O_2 distribution results show that in components from acid compounds with molar masses until C_{39} carbons. This phenomenon increases in the pentacyclic and tetracyclic compounds from ME-a to Me-d. Using a lacustrine fresh water oil sample together with the marine ares ME-a to ME-d in the chart y axis the ratio hopanes/steranos (S/H) versus hopanes/steranes using GC-MS data in the x axis, we can see that the in the lacustrine oil are ordered up in the range 20 to 25 (S/H) and in marine samples this S/H range remain in range 0-5. This work is the first in the literature and represents a major advance in the study of biomarkers acids.

L. Koike, L.M.C. Rebouças, F. de A.M. Reis, A.J. Marsaioli, H.H. Richbow and W. Michaelis,1992, Naphtenic acids from crude oils of Campos Basin, , *Org. Geochem.* **18,** 851

PMo-042
13:30 – 14:40

Standard Samples to Establish Benchmark Metrics and Enable Optimization of Quantitative Proteomics Methods

Sean L Seymour[2], Xu Wang[1], Brian Williamson[1], Ignat Shilov[2], John F MacNamera[1], Christie L Hunter[2], Fadi A Abdi[1]

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Keywords:
iTRAQ, validation, benchmark, standard samples, quantitative proteomics

Novel aspects:
Standard samples designed to enable quantitative workflow benchmarking

Abstract:
We have been working to generate a family of carefully designed standard samples comprised of proteins spiked with known variation into complex mixtures to enable the measurement of performance metrics of quantitative proteomics experiments. Our goal is to generate benchmarks of key measures like precision and accuracy as a function of dominant variables such as analyte concentration and extremity of fold change and then to use these benchmarks as a foundation to compare and improve methods. The first quantitative strategy that is being evaluated using the standard sample set is iTRAQ reagent quantification. First, a control experiment was undertaken to measure the identification rate impact on a constant quantity of just the *E.coli* lysate component (1) unlabeled, (2) 4plex-labeled, and (3) 8plex-labeled, employing extensive replicates. Measuring with a TripleTOF 5600™ system, the average peptide-level identification loss relative to the unlabeled sample was 11% and 24% for 4plex and 8plex, respectively (only 15% loss from 4plex to 8plex). In the current round of the study, the quantification accuracy of the known ratios will be evaluated and false discovery rate of detection of differential proteins will be measured by a target-decoy approach. Once benchmarks have been successfully established, we will use these to improve our informatics methodologies used to analyze isobaric quantitative data, compare performance in multiple laboratories, and compare to other quantitative proteomics workflows using the same samples and metrics.

PMo-043
11:10 – 12:20

Improvement in Mass Measurement Accuracy and Resolving Power Using a New Compensated Fourier Transform Ion Cyclotron Resonance Cell

Nobuyuki Shimura[1], Magnus Palmblad[2], Jens Fuchser[3], Yuri E M Van Der Burgt[2], M Goekhan Baykut[3], André M Deelder[2]

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Keywords:
Resolving Power Increase, FT-ICR-MS, New Compensated Cell

Novel aspects:
A new FTICR cell is tested with a number of realistic use cases

Abstract:

Introduction

A recently described Fourier transform ion cyclotron resonance (FTICR) analyzer cell (Nikolaev et al., JASMS 2011) with dynamic harmonization of the electric fields in the Penning trap, together with improvements in vacuum system, electronics and instrument control and data acquisition software, enables ultrahigh resolving power (>1,000,000) in *m/z* 200-2000 in a 12 tesla FTICR system. We will show the improvements in a number of realistic use cases by comparing the new system with the previous generation instrumentation at the same magnetic field. Examples include the resolution and accurate mass determination of peptides, alignment and hybridization with ion trap data in bottom-up proteomics, urine metabolomics and combinatorial chemistry.

Methods

Urine samples were prepared using standard desalting protocols on C18 solid phase extraction tips and spiked with known concentrations of different analytes. Zebrafish embryos were homogenized, proteins extracted and digested using trypsin. Standard peptides were synthesized from the C- to the N-terminus by Fmoc-based solid phase peptide synthesis with Tentagel S AC as a resin. Peptide mixtures were separated by nanoscale liquid chromatography using a 15-cm 75 um i.d. PepMap C18 (Dionex) column on a Dionex Ultimate 3000 system with a flow rate of 300 nL/min. All FTICR mass spectra were acquired on 12 T solariX systems, with two different cell designs (Bruker Daltonics) . Tandem mass spectra were acquired on an amaZon ETD speed ion trap (also Bruker) .

Preliminary Data

A resolving power that could easily be achieved in the new system with the compensated cell was significantly better than the best that was ever reached in the old system. For instance, three standard peptides of elemental composition $H_{100}C_{73}N_{14}O_{26}S$, $H_{100}C_{75}N_{18}O_{17}S_3$ and $H_{100}C_{62}N_{20}O_{29}S$ at *m/z* 811 (doubly protonated) that could only with difficulty be resolved on the old system with a resolving power of 1,500,000, could easily be resolved to the baseline in the new system with a resolving power in excess of 4,000,000. Two synthetic peptides, with sequences MIM and HTH and elemental compositions $H_{31}C_{16}N_3O_4S_2$ and $H_{23}C_{16}N_7O_5$ respectively, could be clearly resolved. The mass difference between these two peptides is only 0.000469 Da the well-known H_2S_8*vs* N_4O split, which is the smallest common mass difference between any two peptides. These two peptides are also those closest to 400 Da of all possible peptide pairs with this mass difference, and therefore useful for evaluation of instrument resolution at *m/z* 400.

Also for realistic samples such as urine metabolites and complex protein digests such as the zebrafish whole embryo, the practically achievable resolving power on a time scale compatible with liquid chromatography (*i.e.* a spectral acquisition rate of approx. 1 Hz) the new system outperformed the old, especially when internally calibrating each FTICR spectrum using peptides identified in a separate ion trap analysis of the same sample, after chromatographic alignment of the FTICR and ion trap datasets.

PMo-044
13:30 – 14:40

Gas phase ion-molecule reactions of C2H5+ and C2H3+ with C2H2 using low-temperature drift tube mass spectrometer

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Keywords:
ion-molecule reaction, low-temperature Drift tube

Novel aspects:
the low temperature rate constants of hydrocarbon ions and acetylene were measured

Abstract:
The results of a study of the ion-molecule reactions of $C_2H_5^+$ and $C_2H_3^+$ with acetylene will be reported. The association reaction forming $C_4H_7^+$ is important to understanding the ion chemistry in Titan's atmosphere. A few experimental data on association reactions exist for temperatures below the temperature of liquid nitrogen. The thermal and low-temperature (125K) rate constants will be reported. The experiments were carried out using the low-temperature drift tube mass spectrometer.

PMo-045
11:10 – 12:20

Assembly Modulated Metallosupramolecular Polymer Formation Observed by CSI-MS and Other Analytical Methods

Masatoshi Kawahata[1], Kadzuya Hirata[2], Hiroshi Danjo[2], Kentaro Yamaguchi[1]

[1]Tokushima Bunri University, Kagawa, Japan, [2]Konan University, Kobe, Japan

Keywords:
CSI-MS, Metallosupramolecular Polymer, cyclophane, Boron

Novel aspects:
Assembly modulated metallosupramolecular polymer formation of D 3 -symmetric tris (spiroborate) cyclophanes (Twin Bowls) and [Fe (tpy) 2] 2 + complex by adjusting countercharges, K+ or Ba 2 +, was definitely observed by CSI-MS and other analytical methods.

Abstract:
Much attention has been paid to the development of supramolecular polymers for the creation of functionalized nanomaterials, and the development of metallosupramolecular polymers is recognized as one of the most important approaches for the creation of functionalized nanomaterials because these polymers show potential for charge or electron transfer and other optoelectronic characteristics. We have reported the design and preparation of a new class of back to back twin bowls (Twin Bowls) of anionic D_3-symmetric tris (spiroborate) cyclophanes that exhibit molecular recognition behavior via π - and electrostatic interactions at both sides of a symmetry plane. Twin Bowls were readily prepared from 2,2',3,3'-tetrahydroxy-1,1'-binaphthyls (THBs) and boric acid. Equimolar amounts of THBs and boric acid were mixed in DMF at 150 °C to afford Twin Bowls^{3-} $(Me_2NH_2^+)_3$ in 85% yield. Dimethylammonium would be generated by hydrolysis of DMF. In this reaction, almost no signals of other oligomeric structures were detected by ESI-MS analysis. This molecular coupling behavior makes it possible to glue various cationic guest molecules to each other via dual host-guest interaction. Particularly in the case of cationic metal complexes, iterative clathration takes place to form metallosupramolecular polymers. Twin Bowls recognize cationic $[Ir(tpy)_2]^{3+}$ (tpy = 2,2' : 6',2''-terpyridine) complex guests by electrostatic and π interactions to form metallosupramolecular polymer structures in the crystal and solution state. The metallosupramolecular polymer structure of Twin Bowls and $[Ir(tpy)_2]^{3+}$ was unequivocally determined by single-crystal X-ray diffraction analysis and iterative encapsulation of $[Ir(tpy)_2]^{3+}$ by Twin Bowls took place to form one-dimensional array of the guest molecules. In addition, two $[Ir(tpy)_2]^{3+}$ are in van der Waals contact each other through the cavity of Twin Bowls, showing that the spherical cationic guests were effectively glued by Twin Bowls. And from the mearsurements of cold-spray ionization (CSI) -MS, more than 13 molecule-aggregates were observed. We hypothesized that this supramolecular polymerization was caused from the balance of charge between hosts and guests, and changing this balance would affect the degree of polymerization, i.e. we could regulate the metallosupramolecular polymerization (elongation and dissociation) by adding a metal cation as a control factor. By using CSI-MS, we examined the change of metallosupramolecular polymerization degree of Twin Bowls and $[Fe(tpy)_2]^{2+}$ complex when another metal cation (Ba^{2+} or K^+) was added as a control factor. The Metallosupramolecular polymer of Twin Bowls and $[Fe(tpy)_2]^{2+}$ complex showed the ion peak corresponding to Twin Bowls / [Fe (tpy)$_2$]$^{2+}$ complex = 1 / 1. The addition of potassium trifluoromethanesulfonate gave the degree of polymerization increased and the ion peak corresponding to elongated metallosupramolecular polymer of Twin Bowls / [Fe (tpy)$_2$]$^{2+}$ complex = 2 / 3 was observed. Whereas the addition of barium trifluoromethanesulfonate gave the dissociation of the mettallosupramolecular polymer and only the Ba^{2+} ion was observed. These indicate that the polymerization with Twin Bowls and [Fe (tpy)$_2$]$^{2+}$ complex was enhanced by the addition of a potassium cation, whereas the presence of a barium cation disturbed the association of the monomers.

PMo-046
13:30 – 14:40

Clusters of Water, Acetonitrile and Azeotropic Mixtures in Gas and Liquid Phases Studied by Liquid-ionization Tandem Mass Spectrometry

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Keywords:
Clusters of water, Water, Azeotropic mixture, Ethanol, Acetonitrile

Novel aspects:
Liquid-ionization mass spectrometry was applied to clarify clusters existing in gas and liquid phases. Azeotropic mixtures showed the presence of stable clusters which evaporate as they are in the liquid.

Abstract:
Studies of clusters are important for understanding the properties of liquids and the mechanisms of organic chemical reactions in a solution.

A tandem mass spectrometer (Extrel CMS, TMAX 2000) equipped with an improved Liquid-ionization (LPI)[1,2] ion source was used to measure mass spectra of hydrogen-bonded clusters in gas and liquid phases. The voltages applied to various electrode for focusing sample ions affect the peak intensities. Therefore these voltages were adjusted to be appropriate. LPI mass spectral patterns observed by the first mass spectrometer (Q1) are affected only by the voltage applied to the needle tip (where sample ions are formed) and the voltage difference between skimmer-1 and skimmer-2. The influences of these voltages and mass spectral patterns clearly indicate that neither adiabatic expansion nor condensation occur in the LPI mass spectrometer. Namely, LPI mass spectra observed by Q1 indicate information about the composition distributions of clusters (neutral) in gas and liquid phases, that is, observed clusters are already in the liquid.

Mass spectra of water in the air have been measured and shown cluster ions of water, expressd as $(H_2O)_nH^+$, with n up to around 50. The numbers of water molecules (n) are related to the humidity in the air.[3] Mass spectra of pure ethanol (99.5%) showed neat ethanol cluster ions $(C_2H_5OH)_mH^+$ with *m* up to 12 and hydrate ions $(C_2H_5OH)_m(H_2O)_nH^+$ with *m* larger than 8 and n = 1 and 2. Hydrate ions, such as 8-1, 9-1 and 10-1, were always observed, regardless of the quantity of background water. Even when the quantity of water increased slightly, no hydrate cluster ions with m smaller than 7 were observed. Molar ratio (water/ethanol) of the 9-1 cluster is 0.111 and agreed with the molar ratio (0.107) of azeotropic mixture of ethanol-water. The latter data suggest the existence of stable clusters like the 9-1, which evaporate as they are in the liquid, and that is the reason why the azeotropic mixture of ethanol-water exist. Results indicate that ethanol clusters with m = 2 to 7 and hydrates like the 9-1 exist in the liquid phase.

Mass spectra of acetonitrile were affected by the presence of a small amount of water and showed the intense peaks corresponding to 2-0 and 2-1 of $(CH_3CN)_m(H_2O)_nH^+$. Mass spectra of azeotropic mixture of acetonitrile-water showed intense peaks corresponding to the cluster ions of 2-0, 2-1 and 3-1, which are often observed in HPLC-MS when using acetonitrile and water as the reversed phase eluate. Results indicate that these clusters are stable enough to evaporate as they are in the liquid phase.

When using high flow rate (>10 micro-L/h) of the sample solution, mass spectra observed by the second mass spectrometer (Q3) showed larger cluster ions than those observed by Q1. The primary ions must be the ions produced in the ion source and react with the sample vapor flowing into the vacuum. Ion-molecule reaction must occur in the collision chamber (Q2), where multiple collisions should occur to produce larger cluster ions. Mass spectra observed by Q3 indicate the results of ion-cluster reactions.

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PMo-047
11:10 – 12:20

ICP-MS and IR techniques used in metal detection and control of some new synthetized materials with bioactive potential

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Keywords:
ICP-MS, IR, bioactive material

Novel aspects:
This work proposes the investigation of new synthetyzed materials with bioactive potential for bone rapair and reconstruction using ICP-MS and IR as a necessary method for toxic metals determination

Abstract:
ICP-MS is a flexible technique that offers many advantages over more traditional techniques for elemental analysis and it has become a widely used tool, for both routine analyses and for research in a variety of areas. Besides the classical tests of soil, water, food, currently a wide range of biological samples, both solid and liquid can be analyzed by ICP-MS : blood, urine, plasma, serum, fluid interstitial, internal organs, hair, teeth or bones. In many cases it is required to determine not only the total amount of a particular item, but also its chemical form, because it may have a significant impact on the bioavailability, mobility and toxicity of the element .

The research in the field of reconstructive medicine is in a continuous search of synthetic biomaterials, but also of new solutions for prosthetic or regeneration of bones. Synthesis and marketing of improved biomaterials is always required as this automatically leads to an improve in the life quality. In this respect the present work uses two complementar techniques (ICP-MS and IR) in the metal ions detection and control of a new synthetized potassium-phosphate material doped with vanadium ions. Potassium, phosphorus and vanadium are bio-elements required for materials used in bone repair but in suitable amounts. Thus, potassium minimizes the loss of calcium from the body and thus reduces the risk of osteoporosis, providing a healthy skeletal system and vanadium is an active agent in helping to the body metabolism, focusing on the growth of bones and teeth strengthening.

By using ICP-MS technique, 9 samples of V_2O_5-P_2O_5-$K_2O$with various quantities of potassium, phosphorus and vanadium were investigated and suitable/expected amounts of metals were detected : for the sample with the lowest content of vanadium (3 mol%) 308225,184 mg/kg P, 60548,927 mg/kg K and 3292,332 mg/kg V were detected and for the sample with the highest content of vanadium (5 mol %) 135363,798 mg/kg P, 28925,277 mg/kg K and 294601,682 mg/kg V were detected. We applied Total Quant analysis method with external standard of 10 μg/l Mg, Cu,Rh, Cd, in, ba, Ce, Pb, U with a level of certainty of ± 25 %. Such an elemental technique is strongly required in the study of materials with bioactive potential for the detection of impurities and toxic elements ; such materials should be from a biological point of view non-toxic and without filtered products.

IR spectra of the studied 9 samples show for low concentration of vanadium ions the specific bands belonging to the phosphate groups, but at high concentration of vanadim ($x > 10$ mol%) the bands belonging to the vibration of V_2O_5 groups dominates the spectra. In the same time the bands belonging to the phosphate groups are strongly reduced except the specific bands of the short chain phosphate units. This suggest that in the studied materials vanadium acts as network modifier for low concentration ($x < 10$ mol%) but may act also as a network former at high concentration of V_2O_5 .

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PMo-048
13:30 – 14:40

Size-dependent structures of zinc oxide cluster ions studied by ion mobility mass spectrometry

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Tohoku University, Sendai, Japan

Keywords:
zinc oxide, clusters, ion mobility spectrometry, annealing, isomerization

Novel aspects:
Three types of structures of zinc oxide cluster ions have been obtained by ion mobility mass spectrometry coupled with theoretical calculations.

Abstract:
Zinc oxide ZnO is a compound of central interest in the field of functional nanomaterials, such as blue light emitting diode, electrode, and photocatalyst. This compound is also used for cosmetics because of its low toxicity. Clusters of zinc oxide, $(ZnO)_n$ can be regarded as local structure models of nanomaterials or condensed phases, and thus the clusters have been studied both experimentally and theoretically [1,2] . Ion mobility spectrometry is one of the powerful methods to obtain geometrical information of ions. This method utilizes the fact that the interaction of ions with buffer gas depends upon their geometrical structures in an ion drift cell, in which He buffer gas is filled and electrostatic field is applied. The injected ions suffer thousands of collisions in the cell, and the time spent in the cell depends on their collision cross sections with He. Thus the isomer ions are separated depending on their collision cross sections with the buffer gas ; compact isomers pass through the cell faster than the bulky isomers.

In this study, structures of zinc oxide cluster ions $(ZnO)_n^+$ (n = 2-15) have been investigated by ion mobility mass spectrometry coupled with quantum chemical calculations. The cluster ions were generated with laser vaporization of Zn rod followed by reactions with O_2 contained in He supersonic jet. The ions were injected by pulsed electric field into an ion drift cell with kinetic energies of 100-400 eV. The cell was filled with He as buffer gas with a pressure of 0.8 Torr, and an electrostatic field of 7.5 V/cm was applied in order to guide the ions downstream. The cell temperature was cooled down to 170 K by liquid N_2 circulation. Separated isomer ions were then accelerated by pulsed electric fields and analyzed in a reflectron time-of-flight (TOF) mass spectrometer. We thus obtained TOF mass spectra sequentially by scanning the time difference, called "arrival time ", between the injection pulse and the acceleration pulse in the TOF mass spectrometer after the cell. As a result, different isomer ions of various size of clusters were separately detected corresponding to the differences of the arrival times in the two dimensional plots of TOF vs. arrival time. The former corresponds to mass and the latter is proportional to the cross sections. Arrival time distributions can be obtained as slices of the two dimensional plots on arrival time axis.

Three series of arrival time distribution were observed for $(ZnO)_n^+$ depending on the cluster size and the ion injection energy into the drift cell. The fastest series is observed for n = 6, 8-15, which corresponds to cage or tube type structures, from the comparison with density functional theory calculations and estimation of ion mobility from an averaged collision cross section calculation. The second corresponds to ring structures, which is predominantly observed for n< 8. The last series is the zigzag or linear structures, which were only weakly observed at the lowest injection energy of 100 eV in the present measurement. Most of these structures are consistent with the previous theoretical predictions [2] , except for the cage structure at n = 6. Isomerization to stable cages or tubes from other structures was observed as an annealing effect with increasing ion injection energy.

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PMo-049
11:10 – 12:20

Photodissociation dynamics of mass-selected cluster ions examined by fragment-ion imaging

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Keywords:
Photodissociation ; Imaging ; Cluster ; Reaction dynamics ; Reflectron

Novel aspects:
Imaging detection of mass-analyzed photofragment ions from mass-selected cluster ions has been fulfilled using a newly developed experimental setup.

Abstract:
Imaging detection has extensively been applied to neutral species so far for the investigations of photodissociation dynamics. We recently developed a new experimental setup for image detection of mass-analyzed photofragment ions from mass-selected cluster ions, using a conventional angular reflectron time-of-flight mass spectrometer coupled with an imaging detector [1]. In this apparatus, the ion image of fragments can be obtained after mass-selection of parent ions. In the present study, we have fulfilled the ion image detection of fragments induced by photolysis of mass-selected Mg^+-Ar and Mg^+-ICH_3 complex ions, and compared the obtained dependences of recoil angular distributions on laser polarization directions and the obtained kinetic energy release of the photodissociation reaction with previously obtained results.

At first we have observed an ion image of Mg^+ (^2P) fragment after photolysis of a mass selected Mg^+-Ar complex. Mg^+Ar_n were produced by laser vaporization of Mg rod and supersonic expansion of Ar gas. The mass selected Mg^+-Ar ion was photodissociated by irradiation with 4.66-eV laser (the 4th harmonic of a pulsed Nd : YAG laser) in the first drift region of the reflecrton. The Mg^+ fragment ions were reflected with a reflectron and their ion images were detected by a microchannel plate (MCP) with phosphor screen. Amplified electrons were converted to photons at the phosphor screen and finally detected with the CCD camera. From the analysis of the image we obtained the results that the translational energy E_{ex} and the anisotropy parameter β was 604±100 cm^{-1} and 0.98, respectively. From obtained image the Mg^+ ion was dissociated immediately after excitation with a direction parallel to the laser polarization direction, although the selectivity was lower than the pure parallel transition ($\beta = 2$). We successfully indicated that very low fragment-ion velocity on the order of several hundreds of ms^{-1} is detectable by this apparatus. From the observed translational energies we also estimated the binding energy of the Mg^+-Ar ground state as D_0= 1222±124 cm^{-1}. This value is consistent with that reported previously, 1237 ± 40 cm^{-1} [2].

We have also investigated an ion image of MgI^+ fragment after photolysis of a mass selected Mg^+-ICH_3 complex. As was already reported by our group [3], Mg^+ICH_3 has a bent geometry in its ground state. The positive charge is almost localized on Mg, and thus the ultraviolet absorption bands are originated from $^2P \leftarrow ^2S$ transition of Mg^+. Present laser excitation at 4.66 eV corresponds to $3p_z \leftarrow 3s$ transition, in which the transition dipole moment μ is parallel to the Mg-I bond axis. This excitation localized on Mg^+ is followed by predissociation from a C-I bond dissociative state producing MgI^+ fragment ion. Based on these considerations, obtained ring image was explained by the dissociation process after excitation much faster than the rotational period of the complex. Maximum value of the kinetic energy release distribution, 1.24±0.13 eV, which was determined from the observed ring radius was also consistent with the previous result, 1.37 eV [3].

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PMo-050 Chemical digestion for imaging mass spectrometry

13:30 – 14:40

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Keywords:
digestion, NTCB, imaging, TOF-SIMS, MALDI

Novel aspects:
Chemical digestion using NTCB for imaging mass spectrometry.

Abstract:
In recent years, imaging mass spectrometry has received considerable attention, owing to its capability to monitor many molecules at the same time. Mass spectral peaks corresponding to particular molecules can be used as biomarkers in disease diagnosis (1-3). In most cases, matrix-assisted laser desorption/ionization (MALDI) is used to softly ionize the molecules, but this method gives poor spatial resolution. We have focused on time-of-flight secondary ion mass spectrometry (TOF-SIMS), which enables two-dimensional mass spectrometry imaging of components with high spatial resolution (4-6).

Recently we reported a new method for enhancing peptide signals in TOF-SIMS (7). The modification of peptide with 2-nitro-5-thiocyanobenzoic acid (NTCB) achieves high sensitivity for TOF-SIMS detection. Peptides containing only one cysteine at the N-terminus were used as model samples in order to evaluate the ionization efficiency of the NTCB-modified peptides, because peptides with internal cysteine residues give multiple digestion fragments. For the quantitative evaluation of ionization efficiency, an inkjet printing technique was also used to obtain reproducible samples. The TOF-SIMS signal intensity of the parent ion was increased 20-fold through NTCB modification of a peptide with the amino acid sequence CKVASLRETYGDMAD. These results demonstrate that the NTCB method has great potential for detecting the peptides by TOF-SIMS because it has the additional feature of chemical digestion of polypeptides containing cysteine. But little has been reported on the chemical digestion using the NTCB method in imaging mass spectrometry.

The purpose of this study was to improve the NTCB method for measurement of two-dimensional distribution of peptides because this method has a problem of migration of the NTCB-modified peptides. The NTCB method consists of three steps: (step 1) cleavage of S-S bond, (step 2) modification of SH to SCN, and (step 3) hydrolysis of modified polypeptides with base. Among these steps, step 3 has a great influence on the migration since this step involves water treatment of the modified peptides. In the present study we show an advanced NTCB method, that is, this method uses both an ammonia vapor as base and a deliquescent salt in order to keep a trace amount of water on a sample.

Chemical digestion of insulin was demonstrated. We prepared an insulin solution using step 1 and step2. NaCl as a deliquescent salt was then added to the solution, and the solution was deposited onto a Si-wafer by ink jet printing. The Si-wafer was exposed to ammonia vapor at 37 ℃ in moist condition. After the treatment, insulin digest peptides were detected by TOF-SIMS. Comparison of optical and TOF-SIMS images of the printed dots showed that the migration caused by step 3 treatment is negligible. We are planning to apply this advanced NTCB method to imaging mass spectrometry of tissue sections.

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PMo-051
11:10 – 12:20

Enhanced peptide molecular imaging by depositing ice in Time-of-Flight Secondary Ion Mass Spectrometry

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Canon Inc., Tokyo, Japan

Keywords:
TOF-SIMS, imaging, ice

Novel aspects:
This report brings out a quantitative relationship between ionization enhancement and the amounts of the adherent ice on the sample.

Abstract:
INTRODUCTION ; This report presents a method of enhancing ionization of a sample while retaining the original distribution of the sample constituents in time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging. In recent years, mass spectrometry imaging has received considerable attention, owing to its capability to monitor many molecules at the same time. Mass spectral peaks corresponding to particular molecules can be used as biomarkers in disease diagnosis. In most cases, matrix-assisted laser desorption/ionization (MALDI) is used to softly ionize the molecules, but this method gives poor spatial resolution. On the other hand, time-of-flight secondary ion mass spectrometry enables two-dimensional mass spectrometry imaging of components with sub-micrometer resolution. However, one of the major issues in TOF-SIMS is that the ionization probability of components with $m/z > 500$ is extremely low.

In this research, we report on ionization enhancement in TOF-SIMS imaging by a reproducible method of ice deposition onto the surface of biomaterials. One of the three purposes of this report is to bring out a quantitative relationship between ionization enhancement and the amounts of the adherent ice on the sample. The other purposes are to show that the ice deposition method is suitable for mass imaging analysis because of retaining the original distribution of the sample constituents, and to investigate how ice behaves as ionization promotor.

METHODS ; Peptide samples were prepared on a silicon substrate by using a inkjet printing apparatus to assess signal intensities among samples quantitatively.The area of each spot was about 8×10^{-3} mm^2 (diameter 0.1 mm) , and the peptide amount of that was 67 fmol.

Ice deposition onto the sample was carried out in a preparation chamber of TOF-SIMS. After introducing the peptide sample in the vacuum chamber, H_2O gas was discharged into the chamber through a variable leak valve. At this time, by previously cooling the sample to a temperature equal to or lower than a freezing point or a sublimation point of H_2O, H_2O gas that adsorbed on the sample surface turned to ice. The amount of ice adsorbed on the sample surface was estimated by two methods. One method is to use a crystal oscillator sensor, and the other one is to calculate the incident flux of H_2O molecules impacting onto the sample. By using these methods, the intended amount of ice can be deposited on the sample surface. Following ice deposition onto the inkjet printing dots, the protonated parent ion was measured using TOF-SIMS.

RESULTS and DISCUSSION ; In order to investigate the detailed change of the detection intensity of the sample with respect to the amount of the adherent ice, we prepared several dot pattern samples which were covered with various amounts of ice from 0 to 25 ng/mm^2. It was found that the protonated parent ion intensity varies with the amount of ice, and approximately 1 ng/mm^2 of ice gives the highest intensity, which is roughly 10 times the intensity of the original. Comparison of optical and TOF-SIMS images of the printed dots indicates that the constituents diffusion caused by ice deposition is negligible. It means that this method of ice deposition can keep the original distribution of the sample constituents, and is suitable for mass imaging analysis. Experiments using deuterated ice showed that the adherent ice behaves as part of proton source.

From these results, it can be concluded that controlled ice deposition onto the biomaterials surface is effective for ionization enhancement in TOF-SIMS imaging.

PMo-052
13:30 – 14:40

Direct and indirect imaging of secondary metabolites and enzymatic reactions in plant material by Desorption Electrospray Ionization

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Keywords:
Imaging-MS, Plant-analysis, DESI-MS, cyanogenic-glucosides

Novel aspects:
Imaging of enzymatic reactions in plant material, and direct and indirect DESI imaging of plant material.

Abstract:

Introduction

We have developed an imprinting method, termed Indirect DESI imaging, where instead of imaging plant material directly, an imprint is made on a porous Teflon surface which is then imaged by DESI, providing a very robust and simple imaging method.
We have now shown that although plant cells are disrupted during the imprinting process, initiating possible enzymatic reactions, the method still works for compounds which are degraded in these reactions. Indeed, the method can be used to image such enzymatic reactions in selected areas of the plant material. Furthermore, by using a new DESI solvent composition we have also been able to perform direct imaging of leaves which cannot be imaged using standard DESI solvents, due to the presence of a cuticular wax layer on the plant surface.

Methods

Plant material was pressed with porous Teflon and a piece of tissue paper in order to absorb the surplus plant juice. A rubber sheet was added to distribute the pressure and the sandwich was pressed in a vice for a few seconds. The Teflon imprint was dried in a vacuum desiccator and imaged on a custom-built DESIimaging ion source mounted on a Thermo LTQ-XL linear ion trap mass spectrometer.
Results from the imaging experiments were validated by quantitative LC-MS experiments performed on different small sections of the plant material.

Preliminary Data

Indirect DESI Imaging was performed on leaves and petals of *Hypericum perforatum*, showing the distribution of hypericin and hyperforin. As previously reported, hypericin was found to reside in the black glands of the leaves and petals, while hyperforin was found in translucent glands of the leaves and in spots and broad bands on the petals, respectively.
The method has also been applied for imaging of a variety of cyanogenic glucoside (CG) containing plants such as barley leaves, *Lotus japonicus* leaves, sorghum etiolated seedlings and cassava tubers.
Many of these plants contain CGs together with active β-glucosidases capable of degrading CGs upon damage to the tissue. Although the imprinting method inherently causes damage to the tissue it was found to be highly applicable for imaging of the CG without observing significant degradation due to the imprinting process, since the imprinting time can be kept as low as a few seconds and the enzymatic processes are "frozen " when the imprint is dried in a vacuum desiccator. The fact that enzymatic reactions do take place upon damage to the tissue was shown in an experiment where a leaf of *Lotus japonicus* was pressed with a metal ring and imprinted after 20 minutes. Indirect DESI images showed compounds distributed homogenously throughout the leaf (i.e. unaffected by enzymatic reactions) as well as compounds of decreased abundance in the pressed area (substrates of the enzymatic reaction) and newly formed compounds in the pressed areas (products of the enzymatic reaction) .
Direct DESI imaging was performed on leaves of *Lotus japonicus* and cassava tubers, showing elevated levels of CGs in the base of the leaf and near the peel of the tubers, as confirmed by LC-MS. Direct imaging of the leaves was only possible using a special spray solvent mixture. The cassava tubers were cut in 40 µm thick slices on a cryostat and mounted on glass slides, upon which direct DESI imaging using normal spray solvent mixture was quite straight-forward.

PMo-053
11:10 – 12:20

Sub-Micrometer Imaging of Lipids and Trace Elements in Various Cells with ToF-SIMS and Laser-SNMS

Heinrich F Arlinghaus[1], Felix Draude[1], Sebastian Galla[1], Andreas Pelster[1], Martin Koersgen[1], Jutta Tentschert[2], Harald Jungnickel[2], Andrea Haase[2], Andreas Luch[2], Tanja Schwerdtle[3], Johannes Muething[4]

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Keywords:
ToF-SIMS, Laser-SNMS, Cells, Lipids, Elements

Novel aspects:
ToF-SIMS and Laser-SNMS was used to optimize cell preparation techniques and to detect membrane molecules such as lipids, Ag nanoparticles, and arsenic species in different cells with sub-micrometer spatial resolution.

Abstract:
In recent years, molecular imaging with submicron lateral resolution has become of more and more interest for characterizing specialized compounds in biological samples. As an example, nanoparticles (NPs) gain great commercial interest in the medical field due to their high mobility in human tissue. This enables enrichment in specific organs of the human body, which makes them suitable in applications like drug delivery. On the other hand, high mobility of the nanoparticles might result in adverse health effects. Despite broad applications close to the human body, so far, there is only little knowledge of possible toxicity. In this context, the distribution of nanoparticles within cells is of particular interest. Moreover, not only the distribution of nanoparticles but also elemental and molecular cellular distributions such as metabolites and lipids are interesting for medical research.

In this study, we used time-of-flight secondary ion mass spectrometry (ToF-SIMS) and laser-secondary neutral mass spectrometry (Laser-SNMS) to investigate different cells both unexposed and exposed in vitro to silver nanoparticles (AgNPs) and arsenic species. To optimize the analysis, a special silicon wafer sandwich preparation technique was employed ; this entails freeze-fracturing and washing of cell cultures that were grown on silicon wafers. The data showed that during freeze-fracturing, the cell membrane is often stripped from the cell, enabling direct analysis of the interior of the cells on one sandwich wafer and the remaining lipid membrane as a mirror image on the opposite wafer. During analysis, the signal from the nutrient materials was observed to diminish the contrast of the molecular signals in the images. By optimizing the sample preparation and washing procedures, both the contrast and the imaging resolution could be significantly increased due to higher molecular yields and lower background. With these optimization procedures it was possible to detect lipid ions in a higher mass range, especially from those membranes that were stripped from the cells.

Under these optimized conditions, several studies were performed to detect the distributions of trace elements in cells. One study dealt with AgNPs. These particles show antibacterial effects and are therefore applied e.g. in the medical field for wound disinfection or as a coating material for surgical instruments and prostheses. In conjunction with the high mobility of nanoparticles, the antibacterial effects may pose health dangers. In this context the uptake of AgNPs of human macrophages was measured with nanometer-scale resolution. 2 D and 3 D Laser-SNMS images clearly showed that AgNPs are incorporated by macrophages and in part agglomerate to silver aggregates with a diameter of ˜ 3 - 7 µm. In a similar approach, the distribution of arsenic in cells was measured to obtain more information on the reasons why inorganic arsenic proves carcinogenic in humans. A comparison with ToF-SIMS data showed that especially the high elemental sensitivity of Laser-SNMS makes it possible to detect theses trace elements in cells.

PMo-054
13:30 – 14:40

The potential use of reactive DESI to assess oxidative stress in the cell culture

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Keywords:
DESI, oxidative stress, cell culture

Novel aspects:
Examination of oxidative stress in the cell culture using DESI -MS technique

Abstract:
Oxidative stress is a phenomenon involved in many pathological processes such as neurodegenerative diseases (for example Alzheimer's or Parkinson's diseases) or other types of neurodegeneration. It is associated with decreased amounts of antioxidants, and with elevated production of molecules known as reactive oxygen species (ROS) . This may lead to oxidative damage of proteins, lipids, and DNA and, in consequence to apoptosis of the cell.

DESI (desorption by electrospray) is an example of MS imaging approach. This technique is capable of localization and identification of a wide range of substances in biological tissues. DESI utilizes electrospray source to produce charged droplets of organic solvent, which are focused on the analyzed material. After hitting the surface they produce a second generation of charged droplets containing surface molecules. Addition of an appropriate reagent to the carrier solvent results in fast chemical reaction of this substance with molecules of interest localized on the surface. The MS spectra are acquired concurrently, which means that the product of this reaction may be analyzed at once, without additional sample preparation.

Malondialdehyde (MDA) is considered as a marker for lipid peroxidation in living organisms and cultured cells, since it is one of the main products of lipids peroxidation. This compound may be detected with the aid of reactive DESI, where dinitrophenylhydrazine (DNPH) is dissolved in the spray solvent. DNHP is generally used for derivatization of carbonyl compounds, what improves sensitivity of their detection. This substance reacts with MDA to form dihydrazone MDA diDNPH, which might easily be detected by the MS.

For the experiments we applied astrocyte cell culture, since there is a growing number of evidence for their potential role in many processes in the brain, including pathological ones involved in neurodegenerative diseases. Two compounds, widely used in literature that induce pharmacological stress : hydrogen peroxide (H_2O_2) and *tert*-butyl hydroperoxide were utilized to evoke oxidative stress in the cell cultures. The cell pellet, supernatant from the cells homogenized by sonification, and intact cells on the glass surface were analyzed.

To our best knowledge this is the first study to examine oxidative stress in the cell culture using DESI-MS technique. A possibility of rapid measurement of the oxidative stress may provide an opportunity to investigate pathology of CNS, such as drug addiction using cell culture as a model.

The research was supported by the Foundation for Polish Science - POMOST Programme co-financed by the European Union within European Regional Development Fund (POMOST/2011- 3 / 1) and the grant from Ministry of Science and Higher Education no. 3744/B/H03/2011/40 and 3048/B/H03/2009/37.

PMo-055
11:10 – 12:20

Higher sensitivity sub-micron spatial resolution TOF-SIMS bio-molecular imaging using interleaved Gas Cluster Ion Beam (GCIB) sputtering

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Keywords:
Imaging Mss Spectrometry, GCIB, TOF-SIMS

Novel aspects:
The combination of LMIG TOF-SIMS imaging of tissue samples interleaved with GCIB removal of the LMIG-induced damaged layers provides higher sensitivity and optimum spatial resolution imaging for high mass molecules.

Abstract:
Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) has recently evolved as a molecular surface analysis technique with sub-micron spatial resolution. Based on these unique capabilities, applications of TOF-SIMS are rapidly expanding to lipid and metabolite research in medical science, pharmaceuticals, and molecular biology. One limitation in these imaging studies has been the low signal level of molecular ions from higher mass materials, e.g. lipids with molecular weights above 700 Da, when imaging with sub-micron spatial resolution. This is due to the creation of a limited number of secondary ions per square micron from the outermost layer of the sample while the Liquid Metal Ion Gun (LMIG) primary ion beam induces molecular damage in the near surface region. To enhance the quality of molecular ion imaging, there have been several efforts to increase the efficiency, or the secondary ion yield, for high mass ions. These studies include the use of higher energy projectiles [1], cluster ion beam projectiles [2], and the deposition of inorganic or organic secondary ion yield enhancing materials on the sample surfaces [3].

In this paper, we discuss higher sensitivity TOF-SIMS molecular imaging with high spatial resolution using a combination of Bi_3^{++}primary ions from a LMIG interleaved with Ar gas cluster ion beam (GCIB) sputtering. The GCIB beam is used to remove a very thin damaged surface layer after the LMIG acquisition of the maximum secondary ion signal intensity from the outermost surface [4]. This interleaved process can significantly increase the signal intensity while maintaining optimum spatial resolution.

Results will be presented for imaging several lipids from tissue samples using LMIG data from only the outer surface of the sample as well as data acquired with the interleaved LMIG / GCIB protocol. The comparisons of these results will be discussed relative to the ultimate spatial resolution and the molecular ion sensitivity achievable for TOF-SIMS imaging of lipids in tissue samples.

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PMo-056
13:30 – 14:40

Drug and metabolites study in whole body animal by High Definition MALDI imaging

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Keywords:
Imaging, DMPK, MALDI, Ion Mobility, Pharmaceutical Applications

Novel aspects:
Parallel drug and metabolite localization in whole body section tissue samples in the presence of endogenous species separated by IMS.

Abstract:
Introduction

Mass spectrometry Imaging (MSI) is increasingly used in pharmacokinetic studies during preclinical studies. It has been recognized as a complementary technique to Whole-Body Autoradiography (WBA) , which is traditionally used for approval of a drug by the Food and Drug Administration (FDA) agency. The two main advantages of MSI are cost savings compared to radio-labeling of the drug and the absolute confirmation that the drug and its possibly produced metabolites are indeed visualized.

Here, we present the results from a study where two MS based imaging approaches were used to illustrate the spatial distributions of a drug and its metabolites, along with untargeted analysis of endogenous molecules in a whole body section.

Methods

Olanzapine (OLZ) was administrated to rats at 8 mg/kg. Animals were euthanized at 2 or 6 hours post-dose. 30-µm-thick whole-body sagittal tissue sections were mounted on invisible mending tape. A single sagittal section of a whole rat spanned three MALDI plates. CHCA matrix was applied evenly to the sample in several coats using a SunCollect nebulising spray device.

Data were acquired using a MALDI SYNAPT G2 mass spectrometer in both MS and MS/MS mode with tri-wave ion guide optics to separate ions according to their ionic mobility in the gas phase. The obtained data sets were subsequently processed using High Definition Imaging (HDI) MALDI software for detailed image analysis.

Preliminary Data

OLZ is a drug of which its pharmacokinetics[1] have been intensively studied in different animal systems. However, spatial distribution analysis solely by MSI lacks certain, specific information. The two main metabolites of OLZ (m/z 313.15) that have been identified in rat are N-desmethyl OLZ (m/z 299.13) and 2-hydroxymethyl OLZ (m/z 329.14) . Typically, drug distribution analysis by MSI is conducted by using a targeted analysis method for the drug of interest, aiming to achieve better sensitivity and specificity, where the molecular ion is selected by a quadrupole, fragmented in a collision cell and finally the product ions m/z separated before detection. In this study, traditional targeted and untargeted MSI analysis experiments have been explored and contrasted as detailed below.

A first experiment was carried out on the 2 h post-dose tissue section in a multiplex targeted MS/MS, approach where the drug and the two known metabolites were MALDI imaged from a single tissue section.

A second experiment was performed on the 6 h post-dose tissue section in an untargeted MS approach. Also in this instance, both the drug and the two main metabolites were imaged. However, here a vast amount of information is also generated by the ionisation of the endogenous species present in the whole body tissue section.

The final part of the study involved the performance evaluation of isobaric species separation in the gas phase by means of ion mobility separation, which is integrated in the MALDI SYNAPT G2 HDMS instrument, for untargeted MALDI-MS imaging experiments of OLZ and its two main metabolites.

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PMo-057
11:10 – 12:20

DESI-MS imaging of differences between physiological and morphine-treated rat brains.

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Keywords:
DESI, morphine addiction, metabolomics, brain

Novel aspects:
Comparison of brains low MW chemistry between control and morphine exposed brains. Application of DESI-MS technique in complex, comparable analysis.

Abstract:
Drug addiction is one of the growing and still unsolved problems among societies of countries all over the world. Drug abusers are involved in trade of illegal substances and are susceptible to dangerous, infectious diseases. They are also significant source of criminal behaviors. The society bears significant costs of treatment of drug abusers but, despite intense investigations, there is still no effective therapy proposed for the addicts. The modern medicine offers psychotherapy supplemented by pharmacology with antagonists or weak agonists of the selected brain receptors. This strategy is definitely not sufficient, and such therapies usually show low effectiveness. To evaluate and finally develop an effective, open and cheap treatment for drug addicts, the crucial task is to understand molecular mechanisms involved in dependence, tolerance, and withdrawal. Based on our previous experiences in proteomic approach, we decided to use the rat morphine dependence model for the comparison of localization and distribution of low molecular mass molecules in the control, and morphine-exposed brains. Such approach allows for observation and comparison of global changes in low MW molecules among major brain structures between physiological and pathological states. Investigations were performed using DESI ion source (Prosolia, Indianapolis, USA) connected to the Amazon ETD mass spectrometer (Bruker Daltonics, Bremen, Germany) . DESI-MS seems to be one of the most promising, mass spectrometry based, imaging techniques operating at ambient pressure, available since a few years. We examined density and localization maps of the selected, low molecular mass substances in the range of 100-1200 m/z. Mass spectra were taken in the positive and negative modes. For the experimental purposes the rats were given 10mg/kg of morphine hydrochloride once daily (sc.) in days : 1, 3, 5 and 7, what stays in agreement with major morphine dependence models described widely in literature. Additionally, dependence status was verified by precipitation of withdrawal signs by naloxone. Maps were constructed from the frontal sections (ca. 40 micrometers/slice) of the rat's control brains. We compared them with the maps received from brains of rats after morphine injections. Typically, at least 10 density maps from each brain were taken for experiments. Slices were acquired from various areas in an interaural axis from +10 to + 2 mm range (corresponding to Bregma +1.5 to - 7 mm) what allowed for detailed visualization of major structures of the reward system, as well as other structures, clearly visible on the slices, such as hippocampus, striatum, and cortex.

The obtained results bring a new insight into time-dependent activation of brain structures after morphine treatment, and show differences in the low MW range between compared states, what might be useful for further research on the brain areas involved in addiction. Presented findings may also be valuable from therapeutics point of view, showing early mechanisms during development of addiction.

This work was supported by the grants from Polish National Science Center (grants number 3744/B/H03/2011/40 and 3048/B/H03/2009/37) and Foundation for Polish Science - POMOST Program co-financed by the European Union within European Regional Development Fund (POMOST/2011- 3 / 1) .

PMo-058
13:30 – 14:40

High-resolution TOF-SIMS Imaging reveals Multi-layered Barrier Structure of the Stratum Corneum of Skin

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Keywords:
TOF-SIMS, skin barrier, stratum corneum, filaggrin, atopic dermatitis

Novel aspects:
Using TOF-SIMS imaging, we found that skin stratum corneum has at least three distinct layers that have different barrier functions likely corresponding to metabolic process of filaggrin.

Abstract:
Stratum corneum (SC) is the fundamental structure of skin surface to protect the body of mammals from desiccation and foreign insults. SC is a water repellent barrier constituted by corneocytes and inter-corneocyte lipids. Congenital SC barrier insufficiencies, i.e. filaggrin deficiency, are hypothesized to predispose to atopic diseases such as atopic dermatitis, atopic asthma, allergic rhinitis, and food allergy via augmentation of percutaneous allergen infiltration and subsequent sensitization to various allergens. Limitation of analyzing methods, however, hampers detailed analyses of SC barrier structure and barrier function. Here we introduce time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging combining with immunofluorescence imaging to co-visualize SC structure and infiltration of external molecules using mouse skin as a model. Based on the distribution of Na, K and arginine, we temporary divide the SC into three distinct layers : an upper Na^{high} K^{high} $arginine^{low}$ layer (upper-SC) , a middle Na^{high} K^{low} $arginine^{high}$ layer (mid-SC) , and a thin bottom layer of Na^{high} K^{low} $arginine^{low}$ (bottom-SC) . We found that the upper-SC works as a sponge-like layer where several external ions easily infiltrated in. The mid-SC contains higher amounts of free arginine, one of the major degradative products of filaggrin, and prohibited the infiltration of topically applied potassium and hexavalent chromium. In contrast, trivalent chromium infiltrated into the mid-SC but not into the bottom-SC. Interestingly, filaggrin-null SC revealed frequent infiltration of topically applied trivalent chromium into living cell layer, strongly suggesting that focal barrier breakage took place at the bottom-SC in filaggrin-deficient conditions. Our results first demonstrated that SC has at least three distinct layers that have different barrier functions likely corresponding to metabolic process of filaggrin. We anticipate our TOF-SIMS imaging technique to be a starting point for the analysis of SC barrier deficiency in atopic diseases and the evaluation of medicines for skin barrier recovery.

PMo-059 Quantitative MALDI Imaging of a Peptide Pharmaceutical for Drug Distribution Studies

11:10 – 12:20

Nozomi Takai, Yukari Tanaka, Ayahisa Watanabe

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Keywords:
biopharmaceutical, peptide, MALDI, imaging MS

Novel aspects:
This study suggests that MALDI-IMS enables the analysis of the distribution of peptide pharmaceuticals. In addition, the quantitative analysis was achieved by MALDI-IMS.

Abstract:

Introduction

Biopharmaceuticals, such as peptide and antibody drugs, are expected to contribute to innovative drug therapy, and an increasing numbers of pharmaceutical industries are challenging the development of biopharmaceuticals. In drug discovery and development, studies about the distribution of a drug after its administration provide useful information on the mechanisms for the expression of efficacy or toxicity. Therefore, quantitative and efficient methods for the analysis of the distribution of biopharmaceuticals will be required.

Imaging MS (IMS) has been developed to visualize the distribution of drugs or biological molecules on tissue sections without radiolabeling, and recently, several reports have demonstrated the quantitative performance by MALDI-IMS. Thus, MALDI-IMS is expected to enable the quantitative analysis of the distribution of biopharmaceuticals.

In this study, MALDI-IMS was performed on tissue sections of mice administered with octreotide, a synthetic octapeptide analogue of somatostatin.

Methods

Male 8-week-old C57BL/6J mice were injected via the lateral tail vein with octreotide (3 mg/10 mL/kg). At 15 min after dose administration, the liver and kidneys of each mouse were dissected. A half of the liver and the left kidney were frozen to perform MALDI-IMS, while another half of the liver and the right kidney were homogenized with distilled water to determine the concentration of octreotide in each tissue by LC-MS/MS. To perform MALDI-IMS, 10-µm-thick sections were obtained from the frozen tissues, coated with 2,5-dihydroxybenzoic acid (DHB) as a MALDI matrix compound using an automatic vaporizer (ImagePrep, Bruker Daltonics), and then analyzed using a linear ion trap mass spectrometer equipped with a MALDI source (MALDI LTQ XL, Thermo Fisher Scientific).

Preliminary Data

Prior to MALDI-IMS, a standard solution of octreotide mixed with a MALDI matrix compound on the stainless plate was analyzed. In MS analysis, the [M + H]+ ion of octreotide (m/z 1019) was detected with higher intensity in the use of DHB than in the use of α-cyano-4-hydroxycinnamic acid. The doubly charged ion of octreotide (m/z 510) was not observed, unlike in ESI-based analysis. In MS/MS analysis of protonated octreotide, product ions at m/z 914 and 1001 had relatively high intensities. Next, a standard solution of octreotide spotted onto a liver section of a non-dosed mouse was analyzed by MALDI-IMS. A product ion at m/z 914 gave a better signal-to-noise ratio on the liver section than [M + H]+ ion (m/z 1019) or another product ion (m/z 1001). Thus, the m/z 1019→914 transition was used to obtain the octreotide-specific ion images in the following studies.

On the tissue sections of the mice injected with octreotide, the distribution of octreotide was clearly visualized by MALDI-IMS. Furthermore, IMS-based signal intensity of octreotide correlated with the concentration of octreotide in the tissue samples.

PMo-060
13:30 – 14:40

Investigation of Zeolite Matrix for Analysis of Pharmacokinetics Using Imaging Mass Spectrometry with Laser Desorption/Ionization

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Keywords:
Imaging Mass Spectrometry, Laser Desorption/Ionization, Pharmacokinetics, Zeolite Matrix

Novel aspects:
By using zeolite matrix, the ion signal intensity of a drug from a mouse brain section was 1.5 times higher than that using the conventional organic matrix.

Abstract:
Introduction: Acceleration of the processes of new drug development is desired in pharmaceutical industry. Recently, imaging mass spectrometry (IMS) has increasingly got attention as a novel analysis method of pharmacokinetics, which investigates absorption, distribution, metabolism and excretion of an administered drug. Compared to the conventional method, autoradiography, IMS has advantages in measuring time, cost, and ability of distinguishing the drug from its metabolites. However, IMS is inferior to autoradiography in the point of detection sensitivity of the drug[1)]. Therefore, it is important to choose the optimal ionization assisting reagent and its solvent, laser irradiation condition, and so on. The aim of this study is to investigate zeolite matrix for analysis of pharmacokinetics using IMS in order to enhance detection sensitivity. Zeolite matrix is a mixture of an organic matrix and zeolite, which is crystalline aluminosilicate with nanosized cages. It has been reported that zeolite matrix can improve the signal intensities of protonated ion of peptides[2)]. In the present study, ion signal intensity from a brain tissue section obtained from a mouse administered a drug was measured using the zeolite matirix, and compared with that measured using the conventional organic matrix.

Materials and Methods: A mouse (C57BL/6J) was administered buspirone by tail vein injection at a dose of 20 mg/kg. Buspirone is an anxiolytic psychoactive drug. 30 minutes after the injection, the brain was removed and frozen. α-cyano-4-hydroxycinnamic acid (CHCA) and the zeolite (NaY5.6) were mixed in equal amount, and the zeolite matrix was dissolved in 70/30 acetonitrile (ACN) /water at a concentration of 8 mg/mL. The frozen brain tissue was sliced into 2 μm thickness with a cryostat microtome and attached onto a metal sample plate. 1 μL zeolite matrix solution was spotted onto the brain section with a pipette and dried. For comparison, 1 μL CHCA solution in 50/50 ACN/water at a concentration of 10 mg/mL was spotted onto the mouse brain section and dried. A time-of-flight mass spectrometer (Voyager-DE PRO, Applied Biosystems) was used, and the third harmonic of a Nd : YAG laser (GAIA II 30-T, Rayture Systems Co., Ltd.) was irradiated to the sample. 20 points randomly selected in the dried matrix spot, and 100 laser pulses were irradiated to each point. Then, measured 20 mass spectra were averaged. Laser pulse energy was set to 10, 15, 20 and 25 μJ. Under the same conditions, five mass spectra were measured for zeolite matrix and CHCA, respectively.

Results and Discussion: Using both ionization assisting reagents, the signal intensity of protonated ion of buspirone with the laser pulse energy of 15 μJ was the highest. When laser pulse energy was higher than 20 μJ, the signal intensity was decreased with the increase in the laser pulse energy. The signal intensity of protonated ion of buspirone at the laser pulse energy of 15 μJ using the zeolite matrix was 1.5 times higher than that measured using CHCA at the same laser pulse energy. It seems that the water in nanosize cages of the zeolite contributed to the protonation of buspirone. Therefore, it is supposed that the detection sensitivity of buspirone could be enhanced by using the zeolite matrix. In the future work, improvement of the deposition method, the sample preparation method, and so on will be investigated. In addition, the distribution of buspirone in the mouse brain tissue section will be measured with IMS using the zeolite matrix.

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PMo-061
11:10 – 12:20

Imaging the Penetration Behaviour of Topically Applied Drugs into Skin by Desorption Electrospray Ionization Mass Spectrometry

Janina Thunig[1], Rasmus Mortensen[2], Louise Bastholm-Jensen[2], Steen H Hansen[1], Christian Janfelt[1]

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Keywords:
DESI-MS, Imaging-MS, Skin, Creams-and-ointments, In-vitro-studies

Novel aspects:
DESI-MS imaging of the penetration of topically applied substances ; DESI-MS imaging of skin sections

Abstract:

INTRODUCTION

Studying the penetration behaviour of substances into skin is of great importance for toxicologists and for the development of new pharmaceutical products that are delivered into and through the skin. The *stratum corneum*, the outermost layer of the skin, acts as a protective boundary for exogenous substances, preventing them from entering the body. Several substances and formulations can permeate the skin barrier and this effect is used for topical delivery of substances that either need to be delivered locally, or are rapidly metabolized upon oral delivery. In principle there are three different ways of penetration through the skin for exogenous substances : transcellular diffusion through the corneocytes, intercellular diffusion through the lipid sheets between the corneocytes, and penetration of the compounds via perspiratory glands or hair follicles. The importance of the latter pathway has long been underestimated due to a lack of appropriate experimental models, and has sparked scientists' attention only within the last ten years. As an example, for caffeine it was found that 50 % of the assimilated substance penetrated via the hair follicles [1] . The follicular pathway is normally studied by manually closing the single hair follicles with wax, a laborious procedure, and measuring the drug level in blood or a receptor solution. Here we present an alternative qualitative approach for the study of the skin penetration behaviour of topically applied substances, using pig ear skin as an *in vitro* model and desorption electrospray ionization (DESI) mass spectrometry imaging for the detection of drug substances and lipids.

METHOD

All hair is removed from the pig ear and the formulation is applied to the skin for different periods between 0 and 24 hours in order to follow the penetration of the compounds into the skin over time. After the application period, surplus ointment is removed from the skin and the treated area is cut from the rest of the pig ear. For the first time point (0 hours) the formulation is applied and wiped off immediately to determine the signal that is measured from residues of the ointment on the skin. The tissue samples are frozen at -80°C until they are cut into 18-25 µm thick slices on a cryotome, both in vertical and in horizontal direction. The samples are kept at -80°C until DESI analysis. DESI imaging is performed on a Thermo XL Linear Ion Trap mass spectrometer, equipped with a custom-built imaging stage, as described in [2] . Images are recorded with 100 µm resolution. Light micrographs of the samples are recorded before imaging and adjacent tissue slices are H&E stained for comparison with the DESI images.

PRELIMINARY DATA

In negative ionization mode DESI images show the heterogeneous distribution of lipids in the different compartments of the skin. The distribution of hair follicles in horizontal cuts could be pictured in negative ionization mode by following the distribution of cholesterol sulphate which is associated with the hair follicles. Optical images confirmed the localisation of hair follicles. Several drug substances were topically applied to pig ear skin for different application periods, and the distribution of the substances in positive and negative ion mode was recorded. This way the skin penetration behaviour of e.g. ibuprofen and lidocaine was imaged.

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PMo-062
13:30 – 14:40

Platinum Vapor Deposition Surface-Assisted Laser Desorption/Ionization for Mass Spectrometry Imaging of Small Molecules

Tomoyuki Ozawa[1,2], Hirotaka Hisatomi[2], Tetsuro Higashiisokawa[2], Hideya Kawasaki[2], Ryuichi Arakawa[2]

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Keywords:
SALDI-MS, Mass Spectrometry Imaging , Platinum Vapor Deposition

Novel aspects:
Effectiveness of Pt vapor deposition SALDI-MSI on the direct detection of small analytes for printed papers and TLC plates.

Abstract:

Introduction

Recently, a method of coupling MALDI/MS with mass spectrometry imaging (MALDI/MSI) has attracted considerable interest in tissue imaging. However, MALDI/MSI has some problems, (1) the spatial resolution depends on the dispersion size of organic matrix crystals and inhomogeneous crystallization of organic matrix may give poorly reproducible ion peaks at each point, (2) the substrates need to be electrically conductive in order to allow for desorption of the ions from the plate. To eliminate these problems in MALDI/MSI, a method of coupling surface-assisted laser desorption/ionization mass spectrometry with imaging mass spectrometry (SALDI/MSI) using nanoparticles and a nanostructured substrate has been proposed as an organic-matrix-free approach. In this study, we first report Pt vapor deposition SALDI/MSI of small molecules as a solvent-free, homogenous, organic-matrix free and not depend on substrate method.

Methods

The Pt vapor deposition MSI experiments were performed by using a MALDI-TOF MS, AutoFlex III, Bruker equipped with a YAG laser (355 nm) . The Pt deposition onto the target imaging sample was performed by a commercially available magnetron sputtering device (E-1030, Hitachi) . The Pt-deposited sample was mounted onto a standard stainless steel MALDI plate using electrical conductive tape. MSI was conducted in a point-to-point scanning fashion, where the laser beam rasters across a sample surface. Thus, a mass spectrum was collected at each point. Later reconstruction of the 2-D ion maps of the molecules of interest revealed their spatial distributions by using Bruker software (flexImaging) .

Results

We demonstrate the effectiveness of the Pt vapor deposition SALDI/MSI (Pt-MSI) on the direct detection of small analytes without extraction or concentration for printed paper, as well as of various analytes (saccharides, pigments and drugs) separated by glass-backed thin-layer chromatography (TLC) plate.

1. Inkjet printing

The Pt-MSI method was applied to inkjet-printed papers in order to profile the distribution of ink colorant and ink components using a custom inkjet ink formulation of known composition (magenta) on both printed and unprinted papers. The degradation of ink components via UV light was also examined. The distinct optical images were obtained from inkjet printing with magenta on a paper and with the exposure of UV light. We observed several ion peaks in the mass spectra of the printed paper ; magenta ink colourant (m/z = 603) , inkjet ink component (m/z = 172) from the printed region, the UV degradation product (m/z = 188) from the printed region and a paper component (m/z = 393) from an unprinted region. These ion signals on the printed papers were not detected without the Pt vapor deposition.

2. TLC-MS

We compared Pt-MSI with Au-MSI and LDI-MSI (no deposition) for pigments (crystal violet, Rhodamine 123, and Rhodamine B) on the TLC plate. The peak intensities of the pigments are much lower in LDI-MS than when using metal deposition. And using Pt deposition is greater than when using Au deposition.

Pt-MSI can be successfully applied to the imaging analysis of saccharide mixtures (D (+) -glucose, maltose and maltotriose) separated on the TLC plate. In the photograph of a TLC silica plate after the TLC separation, we could not visually confirm the position of the separated saccharides because we did not use colouring reagents. However, the Pt-MSI image can clarify the location of separated saccharides on the TLC plate. In the mass spectra of these spots separated by TLC, the components are sodium adduct ions at m/z 203 from glucose, m/z 365 from maltose and m/z 527 from maltotriose.

This method would be useful in imaging analyses of various insulating materials such as polymers and biological materials.

PMo-063
11:10 – 12:20

Comparison of MALDI Imaging modalities using a Peptide Gel matrix for stem cell implantation

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Keywords:
MALDI Imaging, PET

Novel aspects:
This preliminary feasibility study will compare two MALDI imaging modalities, as well PET, to be used together in this application to stem cell culture

Abstract:
Introduction

Peptide gels are being investigated as a medium for the location and growth of stem cells in vivo. We are developing approaches based on MALDI-MS imaging and positron emission tomography to map the distribution of these gels at the intended site of stem cell growth and to monitor migration of the peptide elsewhere in the organism. The current study aims to assess detection limits for the peptide in tissue samples and to optimise sample preparation to monitor peptide distribution following administration. The peptide is to be radiolabelled for PET imaging, slightly altering its structure. Migration of the modified peptide relative to the unmodified peptide is to be assessed by MS imaging. This provides an excellent opportunity to compare two different MALDI MS instruments, along with testing prototype MALDI imaging target slides.

Methods

Detection limits were measured by applying known concentrations of the peptide to tissue sections, coating with matrix (CHCA) and Imaging with MALDI MS. Fragmentation patterns during MS/MS were determined by analysis of MS/MS peaks. Direct imaging of the peptide (M+H) + ion and MS/MS imaging were compared to assess their dynamic range and which is more appropriate to mapping the high concentrations in the original gel deposited and low concentrations at remote sites. Initial peptide distributions have been mapped by spiking tissues from newly sacrificed mice, flash freezing in liquid nitrogen, sectioning and observing apparent distribution in tissue sections prepared for MALDI imaging . Imaging experiments were performed using a Shimadzu Axima CFR+, along with a prototype Shimadzu MALDI MS, samples were coated with CHCA matrix using a TLC sampler. 3 different target slides were assessed - steel target, ITO coated glass and prototype Shimadzu target slides.

Preliminary Data

Distribution of the injected peptide in murine heart has been measured directly using the (M+H) + peak and MS/MS experiments performed using the 1121 Da parent and observing the fragment at 974 Da. PET labelling of the peptide will use [18F] fluoroacetaldehde which forms a covalent adduct at lysine residues. Activities of up to 287 MBq of the [18F] fluoroacetaldehde labelling agent have thus far been generated in low-activity trials. Limits of detection will be determined and data from MS/MS experiments along with images showing peptide distributions at high and low concentrations will be presented.

PMo-064
13:30 – 14:40

NALDI-IMAGING OF TUMORS: PHOSPHOETANOLAMINE EFFECT ON THE LIPID PROFILE OF A MELANOMA MODEL

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Keywords:
NALDI-imaging, phophoethanolamine, melanoma, phospholipids

Novel aspects:
NALDI- imaging mass spectrometry demostrated to offer the advantages of little or no sample preparation and to show the changes in the phospholipid metabolite of the tumor after drug treatment.

Abstract:
Phospholipids are the main components of biological membranes and there is a special interest in elucidating their role in the treatment of cancer. In fact, as observed before, the tumor progression is accompanied by an adaptive metabolic change showing high levels of *phosphatidylcholines* (PCs) and phosphatidylethanolamines (PEs) . A class of potential synthetic antineoplastic phospholipids such as alkylphospholipids, generally termed as "ether lipids ", acting on phospholipid metabolism, represents promising treatment for cancers. In fact, ether lipids, such as phophoethanolamine (PHOS-S) , has been shown to be able to reduce the synthesis of *phosphatidylcholines* (PCs) in cancer cells interfering with turnover of phospholipids. The aim of this work is the evaluation of changing in the lipid profiles of an animal model of melanoma treated with phosphoethanolamine (PHOS-S) by nano-assisted laser-desorption- ionization imaging mass spectrometry (NALDI-IMS) . For the first time, commercially available nano-structured surfaces are used as substrates for imaging tumor tissue sections of mice.
The implantation of B16/F10 melanoma cells was done in the dorsal of the mice. At the 10th day from the inoculation, the animals were randomly divided into 2 groups : the control group received 0.1 mL water (placebo) and the treated group received 0.1 mL phosphoethanolamine (PHOS-S) solution (5 mg/mL) daily during 10 days. After the treatment, control and treated mice were sacrificed and tumor mass was removed. The tumors were frozen in liquid nitrogen, stored at -80 °C , and subsequently cut in 30 μm sections using a cryo-microtome (CM1850, Leica, Germany) . After cutting each slice was transferred on a NALDI plate provided by Bruker Daltonics. The NALDI plate was heated from its other side by the operator's finger. The quick heating, "thaw mounting ", resulted in an imprinting of the section. While the rest of the tissue was washed off by water rinse (approximately 5 ml) , the imprinting of phospholipids stayed on the NALDI surface. It is worth noticing that any matrix application was needed. The NALDI-imaging mass spectrometric experiments were performed using a MALDI-TOF Autoflex III Smartbeam (Bruker Daltonics, Germany and USA) equipped with a 337 nm laser desorption ion source. The spatial (2D) resolution was set to 150 μm. The imaging experiments were replicated trice on consecutive tissues. The images were created and normalized by FlexImaging software and processed by Biomap software. The identification of the phospholipids was done by LIFT-MS and the lipid searches were performed through the on line database, www.lipidmaps.org. For the first time, it has been demonstrated how a commercially available NALDI™ chip can be used as support for desorption/ionization imaging experiments to differentiate the lipid profile of a human melanoma its fatty capsule. NALDI-MS allowed to identify and map the distribution of the lipid tumor biomarkers. Compared with the fatty tissue, the tumor is mainly characterized by an high abundance of monounsaturated and polyunsaturated phospholipids such as [PC (30 : 1) +H]$^+$ at m/z 706.6, [PC (32 : 1) +H]$^+$ at m/z732.6, [PC (34 : 1) +H]$^+$ at m/z 760.6and [PC (36 : 2) +H]$^+$at m/z 786.6. According with the literature [5] , not only an high abundance of phospholipids was observed in this study but also a significant low presence of triacylglycerols (TAGs) at m/z 895.7 and 897.7. Then, the lipid profile of a melanoma was also compared with the one of the same cancer grown in mice fed with PHOS-S.
The PHOS-S-treated tumor slices were imagined and the phospholipid biomarkers mapped. A significant decreasing in abundance of the lipid tumor biomarkers was observed. The treated tumor also presence different stages of tumor development that can be clearly observed in the NALDI images. The results confirmed the PHOS-S's capability to inhibit the phosphatidhylcholines's turnover.

PMo-065
11:10 – 12:20

An optimized organ fixation technique for imaging and quantitative mass spectrometry for high energy phosphate-metabolites

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Keywords:
metabolomics, imaging mass spectrometry, postmortem degradation, microwave irradiation

Novel aspects:
Employing a microwave fixation technique is essential for precise imaging and quantification of metabolites by mass spectrometry.

Abstract:
Previous studies have shown that MALDI-Imaging MS (IMS) can visualize distributions of diverse metabolic molecules in the biological tissues. On the other hand, a highly comprehensive/quantitative analysis of metabolites by capillary electrophoresisMS (CE-MS) can efficiently complements the IMS technology. However, optimization of animal organ sampling protocol for these metabolomic analyses has remained as a quite critical issue, because major degradation of the metabolites occurs in tissues within a couple of ten seconds after death, especially of high energy phosphate-metabolites. In this study, we have evaluated several sample preparation techniques, namely, a focused-microwave irradiation, in situ freezing (ISF) method, and a postmortem freezing fixation (PMF) with decapitation.
As results, we found that the PMF caused unacceptable autolytic reduction in ATP and increases in ADP and AMP ; absolute quantification by CE-MS revealed that 90% of ATP was broken into downstream metabolites, and therefore, AMP level represents a ten times increase. Furthermore, we found that the micro-wave fixation as the best way for our purpose ; extents of the ATP reduction, AMP elevation, lactate elevation as a results of enhanced anerobic respiration, were much lower in the micro-waved samples compared to in-situ frozen brains. Finally, we revealed that the microwave irradiation of brain samples improved IMS data quality in terms of the number of effective pixels and the image contrast (i.e., the sensitivity and dynamic range)

PMo-066

13:30 – 14:40

A novel method to observe cell surface protein GRP78 expression by time of flight-secondary ion mass spectrometer (TOF-SIMS)

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Keywords:

HepG2, Rotenone, GRP78,scanning electrochemical microscopy (SECM), time of flight-secondary ion mass spectrometer (TOF-SIMS)

Novel aspects:

The cell surface protein GRP78 translocation can be observed by TOF-SIMS imaging technology.

Abstract:

The protein expression level play an important role of living organisms. Traditional detection methods include western blot, enzyme-linked immunosorbent assay, fluorescence resonance energy transfer and flow cytometry. But these methods have some limitations and defect. In this study, we used rotenone which is a specific inhibitor of mitochondrial complex I on human hepatocellular liver carcinoma cell line (HepG2). Rotenone induced cell apoptosis progression and endoplasmic reticulum stress. After 48 hours we observed the endoplasmic reticulum stress associated protein GRP78 expression location change by scanning electrochemical microscopy (SECM). We found that HepG2 cells mitochondria membrane potential were signicant decreases as rotenone concentration increased. The concentration of rotenone in 300nM can induce 5% HepG2 cell apoptosis and 500nM can induce about 10% cell apoptosis. Using confocal microscope to observe GRP78 protein expression location, we found some GRP78 translate to cell membrane and some GRP78 in the endoplasmic reticulum. We built the mass image of GRP78 expression by time of flight-secondary ion mass spectrometer (TOF-SIMS) analysis. All the preliminary results showed that the cell surface protein can be observed by TOF-SIMS imaging technology.

PMo-067
11:10 – 12:20

Imaging Mass Spectrometry of Plant Tissue Slices: Comparison of MALDI-TOF MSI and MALDI-FTICR MSI

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Keywords:
MALDI-FTICR MSI, MALDI-TOF MSI, Imaging mass spectrometry, plant tissue, Arabidopsis thaliana

Novel aspects:
We tried to map small molecules in plant tissue slice using ultra-high-resolution and high-accuracy MALDI-FTICR MSI equipped with in-house ion-source. Also we compared the map with convensional MALDI-TOF MSI results.

Abstract:
Recently MALDI mass spectrometry imaging (MSI) has been a powerful tool to map spatial distribution of molecules on the surface of biological materials. Frequently MSI is applied to animal tissue slices to map various biological molecules on the slice, such as functional lipid molecules, drug metabolites and so forth. However, it's still been difficult to apply MSI techneques to plant tissue because of the difficulties in sample preparation and the existence of secondary or tertiary metabolites. In general, plant tissue is highly water rich and cell wall exits between plant cells, so special sample preparation technique was required to make thin slice of plant tissue. Ultra-high-resolution and ultra-high-accuracy in mass spectrometry are also necessary to identify metabolites contained in plant tissue.

We have developed in-house MALDI ion-source, which was originally developed as a microscopic MALDI ion-source, and it was equipped with Bruker-Daltonics commercial FTICR-MS (Apex-Qe-94T) . In our ion-source, small transparent ITO-coated slide glass was inserted into the original ion-funnel horizontally and tightly focused UV-LASER beam was irradiated vertically from upper long working distance Cassegrain mirror objective while sample specimen can be observed by lower zooming video microscope in real-time. High-precision sample stage was also equipped inside the vacuum chamber and the exact position was able to be controlled by in-house software. All of the MSI experiment sequence, including sample specimen observation with video-microscope, sample stage motion control and triggering MALDI-FTMS run, was also controlled by in-house-build software written with LabView.

We tried to make thin (40 micrometer) slice of quick frozen leaf of Arabidopsis thaliana and the frozen slice was vacuum dried under freezing temperature followed by matrix application using sublimation or neblizer spraying, before dedicated for MALDI MSI experiments. A leaf of Arabidopsis thaliana was first quickly frozen and ice-embedded. The ice-block was then embedded in OCT compound followed by micro-slicing using cryo-microtome under freezing temperature. 40 micrometer thick thin slice of Arabidopsis thaliana leaf was successfully obtained by Kawamoto's method utilizing special adhesive support film during thin-slicing. The thin slice laid on adhesive support film was then put on the cold metal block inside vacuum chamber to be dried under freezing temperature. The dried thin slice on the support film was glued on the small ITO-coated slide glass followed by matrix application using sublimation or neblizer spraying.

We tried to use 2,5-dihydroxy-benzoic acid (DHBA) as matrix and MALDI-FTICR MSI experiment was done in negative-ion mode. Also we tried to use another matrix substance 9-amino-acridine (9-AA) because 9-AA was reported to work as a MALDI matrix of small acidic compounds in negative-ion mode, including typical plant hormones such as Auxin. We performed a lot of try-and-error experiments varying thickness of matrix layer on the dried slices, to find optimized experimental parameters to map small molecules on the plant tissue slices.

Also, the samely prepared specimens were MS imaged using commercial MALDI-TOFMS, (Shimadzu AXIMER performance) , in order to discuss the advantages and disadvantages of MALDI-FTICR MSI against convensional MALDI-TOF MSI in plant physiological studies.

PMo-068 MALDI-MS Imaging Atlas of Mouse Development

13:30 – 14:40

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Keywords:
MALDI-TOF MS, Imaging-MS, mouse development

Novel aspects:
MALDI imaging mass spectrometry is an effective new technology for the discovery of molecular signatures of mouse development.

Abstract:
Background: Imaging mass spectrometry (IMS) using matrix-assisted laser desorption ionization (MALDI) is an effective tool for molecular studies of complex biological samples such as human and experimental animal tissue sections. Distribution maps of multiple analytes directly obtained from tissue sections can be correlated with histological and experimental features. Targeting whole mouse embryos by IMS will cover spatial and temporal molecular arrangements without the need for target-specific reagents, allowing the discovery of novel and diagnostic markers of mouse development.
Materials and Methods: From E12 to E18 days post coitus (d.p.c.) mouse embryos were sacrificed for this experiment. Ten micron thick frozen sections of whole mouse embryos were cut on a cryostat. The sections were transferred to precooled conductive Indium-Tin-Oxide coated glass slides for sagittal and transverse orientation. The *ImagePrep* device was used for this experiment which utilized vibrational vaporization of the matrix with a piezo-electric spray head. The MALDI measurement and image analysis was carried out on a linear Autoflex instrument equipped with a smartbeam-laser and with the flexImaging 2.1 and ClinProTools 2.2 software packages (Bruker Daltonik GmbH, Bremen) . MALDI measurements were done in linear mode in a mass range of 1,000-40,000 Da. The lateral resolution for the MALDI imaging was set to 100 μm. Statistical analyses were carried out using the ClinProTools 2.2 Software. For the statistical analyses, the mass spectra were internally recalibrated on common peaks and normalized on the total ion count. An average spectrum created from all single spectra was used for a peak picking and to define integration ranges. The data set was further analyzed by multivariate analysis such as principal component analysis (PCA) and hierarchical clustering. For the PCA analysis and the clustering across different tissues, the mass spectra were selected on the tissue by assigning representative areas of developing tissue in each stage. Following the MALDI analysis, the matrix was washed off the slides with 70% ethanol and the sections were stained with a modified nile blue staining, scanned at low resolution, and co-registered with the MALDI imaging results. This was also referred by *The Atlas of Mouse Development* (Kaufman MH, 1992) .
Results and Discussions: MALDI imaging in combination with hierarchical clustering allows the comprehensive analysis of the *in situ* mouse development proteome. On the basis of this cluster analysis, classification of complex and dynamic distribution of tissue proteome was well comparable to histological distribution. It is possible to trace development of brain, spinal cord, heart, vascular system, lung, bone, liver, kidney, gut, pancreas, bladder, upper and fore limbs, thymus, pineal gland, and so on. Comparing the IMS atlas of mouse development with the above mentioned histology, it is highly efficacious in understanding embryonic development. Therefore, it must be of particular interest to apply this knowledge into phenotypic screening of genetically mutant mouse to find out aberrant molecular organization in the early stage of the mouse development.

PMo-069
11:10 – 12:20

MALDI imaging of desalted rat brain sections reveals the up- and down-regulation of phospholipids by ischemia

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Keywords:
MALDI mass spectrometry imaging, in situ desalting, brain ischemia, lipidonics.

Novel aspects:
Matrix sublimation/deposition combined with in situ desalting for MALDI-MSI successfully revealed the subtle lipidomic changes in the ischemic rat brain parenchyma.

Abstract:
Conventional MALDI mass spectrometry imaging (MALDI-MSI) studies of ischemic brain sections revealed the changes of phospholipids mostly in their alkalinated form as a result of the ischemic assault. However, the massive change of alkali metal ions in the brain parenchyma following ischemic stroke likely masked the underlying changes of phospholipids following brain infarction. To overcome the interference of massive cation change and to successfully reveal the subtle lipidomic changes by ischemia, MALDI-MSI studies were carried out on the ischemic rat brain sections in which the matrix was applied by vacuum sublimation/deposition following a simple in situ desalting process.

Brain ischemia was surgically induced by permanent middle cerebral artery occlusion (pMCAO) in male Sprague-Dawley rats. Ischemic rat brains were collected 24 hours after pMCAO surgery and cut into 14 μm coronal sections, then collected onto the indium-tin oxide (ITO) -coated slides. After the initial vacuum drying, each brain section was drip-washed with 600 μL of 150 mM ammonium acetate solution to remove the endogenous alkali metal ions. Thereafter, the brain sections were vacuum-dried for another 20 min before applying the MALDI matrix 2,5-dihydroxybenzoic acid (DHB) onto the brain section by matrix sublimation/deposition under vacuum.

MALDI-MSI result showed that the distribution of lysophosphatidylcholine 16：0 (LPC 16：0) in the desalted brain section and that of sodiated LPC 16：0 in the neighboring undesalted section was essentially identical, confirming the suitability of the desalting method for MALDI-MSI application. MALDI-MSI of phosphatidylcholines (PCs) containing both saturated and saturated plus monounsaturated fatty acyl (MUFA) residues showed slight increase over the ischemic brain parenchyma. However, the distribution of PC 16：0/18：1 showed a consistent reduction in the infarcted area. The distribution of polyunsaturated fatty acyl (PUFA) residue-containing PCs revealed laminated patterns in the normal cerebral cortex. Ischemia disrupted the laminated pattern of PC 16：0/20：4, and reduced its abundance as well as the abundance of PC 18：0/20：4. However, ischemia led to the subcortical increase of PUFA-containing PCs containing stearoyl residue, and confined their cortical increase to limited areas. In addition, ischemia caused the consistent decrease of sphingomyelin 18：0 (SM 18：0) with the corresponding increase of ceramide in regions showing moderate to high abundance of sphingomyelin. Together, the results showed that other than the decrease of PCs and the corresponding increase of LPCs, ischemia also heterogeneously increased the abundance of PCs containing mostly stearoyl residue over the ischemic areas. Beyond the changes of PCs and LPCs, the consistent decrease of SM may also serve as a marker for brain injury. The combined imaging results revealed the subtle yet potentially important lipidomic changes through which the effective management of brain ischemia may be based upon.

PMo-070

13:30 – 14:40

Visualization of Transdermal Drug Delivery by 2D-DESI Mass Spectrometry ----- a Novel Approach to Generate Pseudo Cross-sectional Skin Images

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Keywords:

DESI, Transdermal Drug Delivery, Mass Spectrometry Imaging, Tape Stripping Technique, Stratum Corneum

Novel aspects:

A novel mass spectrometric visualization method has been developed that allows the generation of pseudo cross-sectional skin images for fast and non-invasive transdermal drug delivery investigations.

Abstract:

Visualization of transdermal drug delivery is important for cosmetic companies that provide topical products containing mild active ingredients. Fluorescent microscopy, CLSM (confocal laser scanning microscopy), and TOF-SIMS (time-of-flight secondary ion mass spectrometry) have been typically used for those purposes. The conventional methods, however, have a distinct disadvantage in which biopsy samples are required to obtain a cross-sectional drug penetration image across skin. We present in this study a novel mass spectrometric visualization method to generate pseudo cross-sectional skin images for fast and non-invasive transdermal drug delivery evaluations. After applying an aliquot of a topical cream containing 1% of 4-methoxy salicylic acid potassium salt (4MSK) to the inner forearm of a healthy male volunteer, skin samples were collected by the tape-stripping (TS) technique. Using the standardized TS procedure, stratum corneum (SC), the most outer barrier layer of epidermis, was collected as a sheet on an adhesive tape. After collecting several SC layers by repetitive TS operations, two-dimensional desorption elestrospray ionization / mass spectrometry (2D-DESI MS) was utilized to obtain planer distribution information of 4MS (K) penetrated into SC. The collected data was processed by a patent pending method to create a pseudo cross-sectional skin image showing 4MS (K) penetration through SC layers. Potentials and limitations of the approach will be discussed on the presentation.

PMo-071
11:10 – 12:20

Chemical sensor arrays for application in mass spectrometry on surfaces and tissues

Martina Lorey[1], Rabah Soliymani[1], Jari Yli-Kauhaluoma[2], Marc Baumann[1]

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Keywords:
MS-Imaging, mass-tag, microchip

Novel aspects:
We aim to develop diagnostic microchips, utilizing targeted secondary detection MS-imaging. These chips would allow rapid diagnosis for various purposes, regardless the size or abundance of target molecules.

Abstract:
MALDI-imaging mass spectrometry has become one of the most promising analysis technologies, allowing measurements of hundreds of different molecules on surfaces at the same time without disruption of sample integrity. However, like all techniques it has limitations : Very hydrophobic proteins, membrane proteins, high mass proteins and low abundant molecules are hard to measure.
We try to overcome those limitations by applying a new approach which was lately published by Thiery et al.(1). TArgeted multiplex MS IMaging (TAMSIM) is a secondary detection method, which allows the concurrent detection of a basically unlimited number of different target molecules on a tissue surface at the same time. The surfaces are treated with probes containing antibodies, which bind to their particular targets. These antibodies carry chemical tags of a defined mass, which are photodissociated by MS laser irradiation and detected via the time-of-flight analyzer in MALDI-TOF. Thiery et al. showed that simultaneous measurement of Chromogranin A and Synatophysin as markers for Langerhans islets on human pancreas tissues by TAMSIM has a very good analogy with the immunohistochemistry staining of the same tissue section(1). To make this approach multiplex, a library of photocleavable tags with different, distinct masses is synthesized. The mass tags contain an activated acid group which randomly binds to all available amino groups.
We aim to apply the same principle for the development of diagnostic microchips by creating two different microarrays for fluid samples.
The first chip is designed to give solely a yes / no answer for e.g. biomarker detection. Specific antibodies against the desired biomarkers are immobilized in an array on the chip surface, every position with another antibody. The fluid sample is treated with the mass tag and applied on the chip. After incubation and washing a fast MS analysis will show on which spots the tag can be detected, thus stating whether the tagged substrate is bound on that spot.
For the second kind of chip the samples are spotted on the chip surface and immobilized. A mixture of differently tagged antibodies, which are specific against the targets of interest, is applied on the chip surface. After incubation and washing, the chip is screened via MS analysis, and each detected tag is stating a different target molecule on which the specific antibodies have bound.
Due to the specific nature of the mass tags no matrix is needed for desorption and ionization, which dramatically increases the signal to noise ratio. Additionally every antibody can bind several tags, resulting in signal amplification. It would be possible to detect virtually every molecule for which a specific antibody can be synthesized, regardless of the size of the target molecule or its abundance compared to other molecules in the same sample.
These chips would allow rapid diagnosis for various purposes : from research and drug testing to differentiation between cancer types to provide optimal, "personalized " medication.

(1) Thiery et al., Proteomics, 2008, 8, 3725-3734.

PMo-072
13:30 – 14:40

The Sausage Omelette: Breakfast of (IMS) Champions or a Novel Quantitation Tool for Small Molecule Imaging?

Shannon Cornett[1], Katherine Kellersberger[1], Mark R Groseclose[2], David S Wagner[2], Stephan Castellino[2]

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Keywords:
Quantitation in MALDI Imaging, drug imaging, FTMS

Novel aspects:
A novel model for quantitation in small molecule IMS studies

Abstract:

Introduction

Imaging Mass Spectrometry (IMS) has proven to be a powerful technique for the analysis of drugs and their metabolites in tissue. In IMS, success is dependent upon the ability to extract the molecule of interest from the tissue efficiently as well as the suitability of that molecule for IMS analysis. We previously introduced a model for assessment of drug suitability and sample preparation optimization based on the "sausage " model. Here we present the refinement of this approach (sausage omelette) and discuss the application of this model to quantitation in IMS studies.

Methods

Bulk tissue was homogenized then aliquots were spiked with varying levels of a small molecule target and mixed thoroughly with minimal addition of water. Spiked homogenates were used to fill wells formed in an embryonic protein-based support matrix, then frozen at -80 until solid and sectioned at 12 um thickness. Following matrix coating with DHB (30 mg/mL in 50% MeOH) , sample sections were analyzed on a Bruker solariX FTMS (Bruker, Billerica, MA) and data analyzed with the flex Imaging software.

Results:

While determination of relative levels of parent drug and metabolites in tissue is valuable to the understanding of distribution and metabolism, the ability to use these data in the context of drug development, including determination of the efficacy and safety of a drug requires a quantification step.

Due to the nature of the MALDI process, particularly in regard to the ionization of analytes directly from treated tissue, the issue of quantitation is problematic. Here, we show the refinement of an approach previously proposed for optimization of sample preparation (including matrix application) and its value in developing a method for quantitation of target molecules directly from tissue sections.

To assess this model, a number of drugs such as reserpine, verapamil, sunitinib, and terfenadine as well as several early-stage development drugs were spiked into homogenized liver aliquots then injected into a support matrix, frozen and sectioned as tissue. Analysis of these sections shows drug signal from homogenate plugs with no evidence of bleeding into the matrix or separation of the plugs from the matrix during sectioning, indicating that this model nicely mimics dosed tissue. It was also observed that the MS signal response is linear with respect to concentration over more than 2 orders of magnitude. If ion pre-selection and accumulation is performed (CASI) , more than 3 orders of magnitude linearity is observed.

Based on our results, this model shows great promise as a simple method for quantitation in small molecule IMS studies.

PMo-073 To Digest or Not to Digest, That's the Question

11:10 – 12:20

Martin Schuerenberg[1], Christine Luebbert[1], Rainer Paape[1], Janine Rattke[1], Axel Walch[2], Sandra Rauser[2], Michael Becker[1]

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Keywords:
Biomarker identification from Imaging, spatioal proteomics, tryptic digest of tissue, LC-MALDI-TOF/TOF, software to link LC-MS with Imaging data

Novel aspects:
New MALDI imaging method that provides identities plus distributions of more than 100 proteins in tissue sections simultaneously.

Abstract:
Introduction:
MALDI-Imaging Mass Spectrometry (IMS) of proteins in tissue sections combined with Top-Down proteomics represents a powerful approach to biomarker discovery. However, lack of direct identification strategies is preventing its broader use in Proteomics studies.
We have developed a new workflow that combines the spatial information obtained by IMS with the routine identification of proteins from tissue sections by LC-MS. Protein digests were generated by trypsinization on two subsequent tissue sections maintaining the spatial distribution of the peptides. One of the trypsinized sections was analyzed by IMS. Peptides from the other section were identified by routine LC-MALDI-MS/MS. The new "Spatial Proteomics workflow " allows routine identification of more than 100 proteins from tissue sections as well as their spatial distribution at the 50 um level.

Methods:
Fresh frozen rat brain, testis samples and human breast cancer biopsies were sectioned to 10 um slices. Each section was analyzed in parallel by analyzed with and without tryptic digestion. For the digests, two subsequent slices were placed onto one glass slide and a trypsin solution was applied to both of them by supersonic vibration. Tryptic peptides were imaged from one section in a reflector mode MALDI-TOF. The section was coated with CCA matrix. Tryptic peptides were identified by nanoLC-MALDI-TOF/TOF analysis and Mascot searching (Matrix Science) after elution from the second slice. A software tool was developed that grouped the peptide masses by their associated protein and linked it to the peptide masses in the bottom-up image for visual inspection of their co localization.

Results:
We analyzed rat organs using the new Spatial Proteomics approach as model systems, at the 50 um spatial resolution level. In both organs, more than 200 peptides present in the images (i.e., 80 %) were identified by the parallel LC-MALDI analysis and approximately 120 proteins localized. As primary validation tools for the assignment of protein distributions in the tissue sections, the intensity and co-localization of 2 or more peptides and the specificity of mapping the peptide molecular weights to 1 or more proteins were used.
As an extension of the established top-down imaging strategy, this bottom-up Spatial Proteomics approach facilitates the identification and simultaneous localization of a much greater number proteins than was previously possible. Proteins of biological relevance were identified that were previously identified by top-down imaging and top-down sequencing such as [1] thymosin-4 and LCFA-CoA from rat testis and [2] Cystein-rich intestinal protein 1 (CRIP1) from breast cancer biopsies - a novel marker for metastatic cancer.
HER2+/CRIP1+ specified human breast cancer biopsies were analyzed using the established Spatial Proteomics workflow. CRIP1 was identified as one in 150 proteins. Arg-68 was present predominantly in the methylated form and to a lower extent in the non-modified form. Arginine methylation heterogeneity present in CRIP1 was in agreement with the unexplained top-down imaging peak pattern [2] in breast cancer biopsies.
Globally, the Spatial Proteomics workflow provided protein distributions that are typically invisible in top-down images. These included large (4 MDa titin) and membrane associated proteins such as MARCKS ; however, mostly cytosolic, nuclear and cytoskeleton associated proteins were identified. The method may also be useful for protein imaging and identification from FFPE tissue in which top-down analysis is made impossible by protein crosslinks and which is largely accessible from tissue banks.

1. Lagarrigue 2011 MCP 10 : M110.005991
2. Rauser 2010 JPR 9 : 1854-63

PMo-074
13:30 – 14:40

Rapid assessment of drug and metabolite distribution in whole animal tissues via accurate mass imaging

Arnd Ingendoh[1], Cristine Quiason[2], Katherine Kellersberger[1], Brian Dean[2], Sheerin Shahidi-Latham[2]

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Keywords:
Imaging, MALDI, FTMS, metabolite, accurate mass

Novel aspects:
Identification of drug and metabolites in whole body tissue sections by rapid accurate mass full scan imaging.

Abstract:
Traditional imaging approaches relying on accurate mass measurements typically require long scan speeds that result in lengthy acquisition times and hefty file sizes. For this reason, applications toward the assessment of whole body drug distributions have been limited. New methods allowing for ion accumulation prior to FTICR detection, along with online data reduction algorithms, have significantly decreased unnecessary overhead, thus considerably improving the overall approach. In this study, rapid imaging of whole body tissues with accurate mass, high resolving power and high dynamic range by MALDI FTMS in broadband mode were successfully obtained in a significantly shorter time frame and allowed for the rapid assessment of drug and metabolite.
Male Sprague-Dawley rats (n= 3) were administered a single 30 mg/kg PO dose of Olanzapine euthanized at 2, 6, and 12 hrs post dose, and flash frozen. Whole-animal carcasses were sectioned at 20 micron thickness and whole-body sections were transferred to MALDI target plates using double-sided tape. A solution of alpha-cyano- 4 -hydroxycinnamic acid (CHCA) matrix (5 mg/ml, 50/50 (v/v) Acetonitrile/H 2 O) was spray coated onto tissues using the automated HTX Imaging sprayer. Full MS spectra from m/z 140-1000 were acquired on a 7.0T SolariX FTMS system (Bruker) equipped with a dual ESI-MALDI source employing smartbeam-II technology. Data were processed and images were extracted using FlexImaging software (Bruker) .
High resolution (resolving power of 150,000 @400 m/z) and exact mass (< 2 ppm) imaging data were obtained in full scan MS mode over the m/z range of 140 to 1000 using the 7 Tesla FTMS (Bruker) . Exact mass (+/-0.002 Da) 2 D images of drug showed unique distributions across the whole-body sections. Each whole body section collected comprised of roughly 24,000 pixels and with the acquisition rate of 0.8 seconds/pixel took 5 hours to acquire. The large sample introduction plate and fast acquisition capabilities of the 7 Tesla FTMS made it an ideal platform for rapid whole body mass spectrometric imaging (WB-MSI) . This study demonstrated the advantages of a fast acquisition approach for whole body imaging because it allowed for the acquisition of a full MS spectrum within several hours, effectively enabling a full data set representing a PK time course to be obtained over a course of a few days rather than weeks by traditional approaches.

PMo-075
11:10 – 12:20

Development of MALDI-MS Imaging of Metabolite and Protein in a Single Tissue Section

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Kyushu University, Fukuoka, Japan

Keywords:
mass spectrometry imaging, metabolite, protein, MALDI-MS

Novel aspects:
Our proposed MALDI-MSI system could visualize spatial distributions about both proteins and metabolites on the same tissue section.

Abstract:

Background

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is a remarkable new technology that enables us to determine the distribution of biological molecules in tissue sections by direct ionization and detection. This technique is now widely used for analysis of biomolecules such as proteins, lipids and drugs, with high sensitivity. Previously, we attempted to apply this technique to low-molecular-weight metabolite imaging using 9-aminoacridine (9-AA) as a matrix. As metabolites represent the compound-level phenotype of information via gene expression and translation, the metabolite imaging technique is expected to be a potential tool to systematically understand the biological processes. On the other hand, an integrated analysis of multiple layers of biomolecules has received much attention to get a better understanding of the entire biological system. Since expression levels of enzymes in some tissues reflect metabolite pattern there, integrated information of spatial distributions of both metabolites and proteins in the same tissue section will be a breakthrough for unraveling complex biological systems. In this study, we developed a MALDI-MSI technique for simultaneous analysis of the multiple biomolecules, proteins and metabolites, in a single tissue section.

Results

Normal rat brain tissues were coronal sliced at 10-μm thickness with a cryostat-microtome and thaw-mounted onto a stainless plate. First, we analyzed the distributions of metabolites in the tissue section. A matrix solution (10 mg/mL 9-AA in 100% methanol) was sprayed to the section using an airbrush. Single reflectron-type MALDI-TOF-MS (AXIMA Confidence, Shimadzu) was used in negative ion mode for metabolite imaging. More than 100 metabolite peaks were detected, and such peaks could be visualized with their unique distributions. By the MS/MS analysis using quadrupole ion trap-type instrument (AXIMA QIT, Shimadzu), we identified about 30 metabolites, including nucleotides, cofactors, phosphorylated sugars, amino acids, lipids, and carboxylic acids. After metabolite imaging, the tissue section was washed twice in 70% ethanol for removing 9-AA, lipids, salts and other contaminants, and once in 100% ethanol for fixing protein. The washed sample was subjected to direct tryptic digestion of proteins on tissue sections (*in situ* digestion). Trypsin solution (0.2 μg/μL in 10 mM ammonium hydrogen carbonate) was spotted onto the tissue section using chemical ink-jet printer CHIP-1000 (Shimadzu), and then 2,5-dihydroxy benzoic acid (25 mg/mL in 0.1% TFA/60% methanol) was spotted on the same point. The tissue sample was analyzed by MALDI-MS (AXIMA QIT) in positive ion mode for peptide MSI, and detected peptides were identified by MS/MS analysis. The MS/MS spectra obtained were submitted to a MASCOT (Matrix Science) database search engine. Spatial distributions of 30 peptide peaks were successfully visualized by the sequential MALDI-MSI after metabolite analysis and several peaks were identified.

Conclusions

The present multistep MALDI-MSI technique enabled to visualize the distribution of both metabolites and proteins on the same brain tissue section for the first time. Overlay of the data from a serial MALDI-MSI allowed the comparison of their relative abundance or spatial localization unambiguously. Although it is still primitive, we believe that our proposed strategy integrating the spatial information of biomolecules in two different layers will strongly contribute to the advance in biological and pharmacological researches.

PMo-076
13:30 – 14:40

Automated Tissue State Assignment for High Resolution FTMS MALDI Imaging Data

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Keywords:
Imaging, MALDI. FTMS, hierarchical clustering

Novel aspects:
First report of hierarchical clustering of MALDI-FTMS imaging data

Abstract:
Interpretation of MALDI imaging data can be tedious, especially if many peaks are present. Hierarchical clustering is a technique that allows clustering of mass spectra based on their overall similarity. The technique is already used for the fast and concise semi-supervised segmentation of MALDI-TOF imaging data (Deininger et. al, J. Proteome Res 2008, 7 (12) : 5230- 6) . Compared to MALDI-TOF spectra of proteins the clustering of FTMS data is more challenging, mainly because FTMS spectra have many more peaks. In this proof-of-concept study several parameters for the hierarchical clustering such as distance and linkage methods and missing value treatment were evaluated and their results compared in respect to computational performance and analytical results.
MALDI imaging data from several model tissues such as mouse kidney, brain, and pancreas were recorded on a FTMS mass spectrometer (Solarix, Bruker Daltonics) . For each mass spectrum a peak list was generated. The peak lists were binned into one bucket table with a custom C# program. Clustering was calculated with R. Different parameters for the distance (such as Euclidean, Correlation, Manhattan) and linkage (Ward, Complete, Average) were used for the calculation, for the Euclidean distances data were also pre-treated with Principal Component Analysis (PCA) . Segmentation maps were reconstructed from the clustered data and compared to the anatomy of the tissue sections.
The data suggest that different distance and linkage methods lead to overall similar segmentations of the dataset, which were in good agreement with the anatomy of the samples. In the kidney, the cortex, medulla, and renal pelvis were clustered as the main tissue structures by all approaches. The same was true for the anatomical regions in the brain. This suggests that the differences in the anatomical regions were significantly larger than random pixel-to-pixel variations, thus leading to a robust clustering result. Differences were observed in the details : Ward linkage led to dendrograms that were easiest to interpret, since they resulted in larger clusters at the top end of the dendrogram. While ward linkage resulted in the clearest segmentation it was computationally not feasible to use this on larger datasets. Correlation was found to be a very appropriate distance method that could be applied without normalization of the data. The preliminary data suggests that since the clustering was robust in the observed cases, it is feasible to select the parameters according to the computational requirements, which can be different for datasets of different sizes. Generally the clustering was applicable to FTMS data and allowed a concise segmentation of the datasets.

PMo-077
11:10 – 12:20

An Investigation of Spatiotemporal Metabolic Behaviors in Response to Pathological Progress using Multiple Mass Spectrometry Techniques

Miho Irie, Yoshinori Fujimura, Daiki Setoyama, Mayumi Yamato, Daisuke Miura, Hiroyuki Wariishi

Kyushu University, Fukuoka, Japan

Keywords:
LC-MS, mass spectrometry imaging, metabolomics

Novel aspects:
Combination of MALDI-MS imaging and LC-MS-based matabolomics would visualize drastic changes in spatiotemporal metabolite distribution of MCAO brain.

Abstract:

Background

Metabolomics, the measurement of global endogenous metabolite profile from a biological sample under different conditions, can lead us to an enhanced understanding of disease mechanisms and the discovery of diagnostic biomarkers. Mass spectrometry (MS) coupled with preseparation techniques such as liquid chromatography (LC-MS) or gas chromatography (GC-MS) is a conventional strategy for metabolomics. These techniques analyze hundreds of metabolites simultaneously ; however, they have drawbacks in analysis of tissue samples due to a requirement of metabolite extraction, which causes the loss of information on spatial localization of the metabolites. To better understand the complex biochemical processes in living organisms requires not only the elucidation of the molecular entities involved in these processes but also their spatial distribution within the organism. Recently, we developed *in situ* metabolomic imaging technique by using matrix-assisted laser desorption ionization (MALDI) -MS that can visualize the localization of a broad range of metabolites simultaneously. The MS imaging (MSI) enabled to trace spatiotemporal metabolic dynamics in the brain of transient middle cerebral artery occlusion (MCAO) rat during infarct formation after ischemia-reperfusion. Although several distinctive changes during pathological progress were observed, detailed metabolic dynamics still remained unclear because of a low coverage of detectable metabolites. Herein, we performed additional LC-MS analysis to understand more comprehensive metabolic behaviors in the brain of MCAO rat. This data together with MSI data was analyzed for characterizing detailed metabolomic response to pathological alterations.

Results

To investigate metabolic variance after reperfusion following 1 h of MCAO, we used rat brains at different time intervals after ischemia-reperfusion (0, 3 and 24 h) . For comparing the metabolic state in ischemic and contralateral hemispheres of MCAO brain, tissues were coronally sliced at 10-μm thickness with cryostat and then subjected to MSI for visualizing the spatial distribution of metabolites. On the other hand, metabolites extracted from three different regions such as whole cortex (CTX) , hippocampus (HI) and corpus striatum (CPu) , were measured by LC-MS. Our previous MSI data of MCAO rat brain indicated that drastic spatiotemporal metabolic changes were occurred in the central metabolic pathway. Holistic evaluation of metabolic profile in each region was firstly investigated by principal component analysis using LC-MS datasets. These results suggest that metabolic states were clearly different between contralateral and ischemic hemispheres or among three representative compartments (CTX, HI and CPu) . In ischemic hemisphere, significant metabolic changes at CTX and CPu were observed after reperfusion, while metabolic state of HI was relatively stable. These observations indicate that metabolic profiling with spatial information is indispensable to better understand the mechanism of disease progress in MCAO brain. Indeed, region-specific metabolic behaviors in amino acid metabolism, TCA cycle and nucleotide metabolism were shown by hierarchical clustering analysis. In each specific region (three compartments and two hemispheres) , data correlations between MSI- and LC-MS-based metabolite analyses were slightly low (averaged R^2=0.24) , but remarkable changes such as citrate and N-acetylaspartate were fully appreciated in both analytical platforms (R^2=0.96 and R^2=0.89, respectively) . These findings raise the possibility that only a single LC-MS analysis in small tissue compartments may cause an underestimation of metabolic changes in the tissue. A combination of different analytical platforms (MSI : spatial information but low coverage ; LC-MS : high coverage but loss of spatial information) enabled us to visualize more comprehensive metabolic behaviors in the tissue micro-regions. Details about metabolic changes and its biological significance in MCAO brain will be discussed in the session.

Conclusion

Our proposed multiple mass spectrometric platforms revealed for the first time detailed metabolic behaviors after reperfusion in MCAO brain. Although this is still primitive, further development of systematic analytical methodology for data integration will contribute to the advances in biological and pharmacological researches.

PMo-078
13:30 – 14:40

Imaging Hair Analyses for Drugs: A New Perspective to Investigate Drug Use History

Tooru Kamata[1], Noriaki Shima[1], Akihiro Miki[1], Kei Zaitsu[1], Munehiro Katagi[1], Michiaki Tatsuno[1], Toyofumi Nakanishi[2], Takako Sato[2], Hitoshi Tsuchihashi[2], Koichi Suzuki[2]

[1]Forensic Science Laboratory, Osaka, Japan, [2]Osaka Medical College, Osaka, Japan

Keywords:
imaging, hair, drug, MALDI, forensics

Novel aspects:
Images of drug-positive bands on the longitudinal section of hair shafts, which can provide chronological information of drug use, were obtained by MALDI-TOF imaging mass spectrometry.

Abstract:
Amphetamine-type stimulants (ATS) represent the most prevalent and potent drugs of abuse. Such drugs ingested into the body circulate in the bloodstream, and trace amounts of the drugs are deposited in the hair follicles and thus incorporated into the hair shafts. This paper presents the direct detection of ATS in hair and its imaging by MALDI-TOF-MS, with careful optimization of sample preparation. Preliminary experiments were performed using human hair shafts spiked with the over-the-counter drug methoxyphenamine (MOP), as an ATS analog, at various concentrations. Method development was further carried out using hair specimens from volunteers who took MOP (e.g., 50 mg/day x 5). A longitudinal section of a lengthwise-cut single human hair shaft was directly analyzed by using the MALDI-TOF mass spectrometer autoflex speed (BrukerDaltonics) which utilized a Smartbeam™-II solid-state laser, after deposited with α-cyano-4-hydroxycinnamic acid matrix using an ImagePrep™ (Bruker Daltonics). Although lengthwise-cutting of a single hair shaft was laborious, this was necessary for effectively detecting ppm-level drugs incorporated in hair. Images of drug-positive bands on the longitudinal section of hair shafts, which can provide chronological information of drug use, were obtained by monitoring analyte-specific product ions in the selected reaction monitoring mode. To further confirm the identity of drugs detected by MALDI-TOF-MS, the analyses of drugs and metabolites in 1-cm segments (from the root side to the tip side) of these hair specimens were carried out by a conventional LCMS-MS procedure.
Thus, imaging mass spectrometry can offer a new perspective in the analyses of drugs in hair : "imaging hair analyses for drugs".

PMo-079
11:10 – 12:20

Easy ambient sonic-spray ionization mass spectrometry imaging (EASI-MSI).

Dragos Lostun[1], Mario F Mirabelli[1,2], Tanam S Hamid[1], Alexander Chramow[1], Demian R Ifa[1]

[1]York University, Toronto, ON, Canada, [2]University of Calabria, Cosenza, Italy

Keywords:
Mass spectrometry imaging, Spray-based, Ambient ionization.

Novel aspects:
Initial characterization of easy ambient sonic-spray ionization mass spectrometry imaging (EASI-MSI) is presented.

Abstract:
Ambient ionization methods (AIM) allow for the examination of surfaces in their native conditions at atmospheric pressure with minimal or no preparation. Spray-based AIM such as desorption electrospray ionization (DESI) and desorption atmospheric pressure photo-ionization (DAPPI) have been successfully applied to mass spectrometry imaging (MSI) . In DESI, a spray of charged droplets creates a thin film on the surface which dissolves the analytes. Subsequent primary droplets hit this film forming secondary microdroplets which then undergo the classic electrospray mechanism of ionization. In DAPPI, a heated jet of mixed nebulizer gas and spray solvent vapor is directed to the sample and promotes thermal desorption of the analytes which then undergo the classic gas-phase mechanism of photoionization. Easy ambient sonic-spray ionization (EASI) is also a spray-based method. It has been applied to direct analysis of inks for document examination and in characterizing organic compounds separated on thin-layer chromatography plates. No voltage or heating is required by EASI. The ionization of the sample is achieved via sonic spray ionization (SSI) assisted by a high-velocity gas flow. Although chemical images have not been presented by EASI, its potential for MSI is implied. We report the initial characterization of EASI-MS for mapping the distribution of (i) inks in printed patterns on paper, (ii) biomolecules from tissues, and (iii) drugs separated by thin-layer chromatography. Preliminary results showed that chemical images can be easily obtained using no voltage DESI. However, the high-velocity gas flow and the solvent flow rate required by EASI represent challenges to attain stable signals and good lateral resolution.

PMo-080
13:30 – 14:40

Optimization of conditions for acetylcholine detection in the nerve tissue sections by imaging mass spectrometry

Ikuko Yao[1,2], Yuki Sugiura[2,3], Nobuhiro Zaima[3], Mitsutoshi Setou[3], Seiji Ito[1]

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Keywords:
imaging, brain, spinal cord, acetylcholine

Novel aspects:
We successfully reconstructed acetylcholine distribution in mouse brain and spinal cord tissue sections by MS/MS imaging.

Abstract:
Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) provides the means for analysis of a wide variety of biomolecules. This technology known as imaging mass spectrometry (IMS) provides us a novel opportunity to visualize diverse types of molecules directly on tissue surfaces without the need for target-specific reagents such as antibodies. As most previous IMS studies analyzed the relative abundances of larger metabolite species, it is important to expand its application to smaller molecules, such as neurotransmitters. Here we applied IMS technique to visualize neurotransmitter distribution in the central nervous system (CNS) .

We chose acetylcholine (ACh) and examined both single MS imaging and MS/MS imaging for ACh. Monitoring ion transition by MS/MS measurement improved the S/N ratio of the ACh-derived signal by eliminating matrix interference. Previous studies reported that collision-induced dissociation of ACh yields a major fragment ion at *m/z* 87 arising from the loss of trimethylamine. Therefore, we compared the S/N ratio and sensitivity of ACh-derived signals between single MS measurements (ion at *m/z* 146) and MS/MS measurements (ion at *m/z* 146 > 87) . For both measurement results of 1 nM ACh standard measurements by MS/MS and single MS measurements, the spectra obtained from the sample containing spots exhibited clear ACh-derived signals, where as those from blank DHB spots only exhibited matrix-derived background peaks. In particular, in the single MS measurement, a DHB-derived peak observed at *m/z* 146 interfered with the detection of ACh in the MS mode. Conversely, there was no observable matrix interference at *m/z* 87 in the product ion spectrum of *m/z* 146. As expected, this advantage offered an excellent S/N ratio that is superior to that of the single MS detection, even for the measurement of a trace sample concentration.

We also tried to directly detect neurotransmitters and visualize their localization in the tissue samples. In MS/MS imaging, the transmitter could be visualized with a high S/N by elimination of matrix interference. In the MS analyses of mouse spinal cord, reconstructed ion intensity maps for ACh ion showed that the ion which corresponds to ACh distributed both inside and outside tissue section, suggesting that containing matrix derived chemical noise. Tandem MS solved the first problem of the sensitivity. In the tandem MS-imaging analysis of ACh in the mouse spinal cord, the signals were detected around the region containing rich motor neurons. Similarly, we analyzed mouse brain sections with tandem MS imaging. In comparison with the previous reports, it was revealed that ACh was localized in the region abundant in acetylcholinestelase.

These results show that ACh is the suitable molecular species to detect in positive ion detection mode by IMS, and that tandem MS imaging is useful for the analyses of tissue distribution of ACh in the CNS tissues.

PMo-081
11:10 – 12:20

MALDI-IMS - The challenge of on-tissue digestion, a comparison of different protocols to improve imaging experiments

Hanna C Diehl, Julian Elm, Judith Baronner, Helmut E Meyer, Corinna Henkel

Medizinisches Proteom Center, Ruhr University Bochum, Germany

Keywords:
MALDI-IMS, Imaging, on tissue-digest, ImagePrep

Novel aspects:
Preliminary results reveal interesting differences concerning the spatial resolution between different drying times of tissue samples.

Abstract:
Abstract
Objective : Matrix-assisted laser desorption ionization (MALDI) -Imaging mass spectrometry (IMS) has become a powerful and successful tool for biomarker detection[1] and drug development. Depending on the research question investigators are trying to go beyond the exclusive visualization of the spatial distribution of potential markers or areas of interest. Their focus lies on the identification of the unknown components and hence increased efforts have been made to develop and improve existing methods.

Current on tissue trypsin digestion techniques in MALDI-IMS are either done via spray coating or droplet deposition of trypsin solution[2,3] and/or matrix solution respectively. Both can be performed either in a manual or automated manner. The latter allows greater confidence of inter-run reproducibility, however the entire process from sample collection to data analysis is influenced by a number of more or less controllable and uncontrollable factors.

Design and Method : The overall goal of this study was to test and compare various protocols of tissue digestion in order to improve the quality and reproducibility of MSI data. Frozen and freshly prepared mammalian tissue samples were automatically sprayed with trypsin and matrix using the ImagePrep device from Bruker Daltonics. Subsequent measurements were carried out with an UltrafleXtreme instrument (Bruker Daltonics) . The different experiments were carried out varying the trypsin incubation times/drying phases, the matrix, the raster width of the laser and the setting of the laser attenuator.

Results : Preliminary results reveal interesting differences concerning the spatial resolution. But due to the fact that the experiments are still ongoing at the time of abstract submission, a final conclusion cannot be drawn.

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Acknowledgement
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PMo-082
13:30 – 14:40

Molecular detection of breast cancer-related phosphatidylinositol by high-resolution imaging mass spectrometry

Masahiro Kawashima[1], Noriko Fukao[2], Minoru Suzuki[2], Taka-aki Sato[2], Koichi Tanaka[2], Nobuko Kawaguchi-Sakita[1], Masahiro Sugimoto[1], Takayuki Ueno[1], Kazuya Terasawa[3], Gozo Tsujimoto[3], Kazuharu Shimizu[3], Masakazu Toi[1]

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Keywords:
imaging mass spectrometry, breast cancer, phosphatidylinositol, PI 3 K pathway, palmitic acid

Novel aspects:
This high-resolution imaging mass spectrometry analysis for breast cancer tissues revealed the PIs reconstruction in breast cancer cells which would affect the PI 3 K or arachidonic acid pathway.

Abstract:
Breast cancer is the common cause of death among Japanese women and its incidence is rapidly growing for the last few decades. Several studies showed the high-fat diet and obesity increase the risk of breast cancer, and suggested that palmitic acid (16：0) and a-linolenic acid (18：3) could affect the breast cancer risk. Furthermore, the phosphatidylinositol-3 kinase (PI 3 K) pathway is frequently mutated in the breast cancer. Its abnormal activation is related to the growth, survival, and resistance of tumors to conventional chemo and radiation therapy. Thus, the blockade of this pathway has a great concern as an emerging target for breast cancer therapy. Although, the composition of phosphatidylinositols (PIs) altered in cancer tissues, its significance or impact on PI 3 K pathway remains poorly understood. The objective of this study is to investigate the phospholipid distribution in breast cancer tissue by the imaging mass spectrometry and find out the new pathological and biological features in terms of the PI and their metabolism.

In this study, we obtained breast cancer tissues from two patients who were diagnosed as the postmenopausal triple-negative (ER-, PgR-, Her 2 -) breast cancer. The tissues were obtained during surgery, quickly frozen, and stored at -80°C until analysis. The serial sections were prepared from each sample and attached onto the indium tin oxide coated glass slides. Every section was coated with 9-aminoacridine (9-AA), which was used as the matrix for matrix-assisted laser desorption/ionization mass spectrometry (MALDI/MS). The 9-AA was mechanically evaporated for positive and negative imaging MS analysis. The imaging mass spectrometry was performed by the high-resolution mass spectrometer (AP-MALDI, 10um spacial resolution, Shimadzu Corp., Kyoto). All imaging data were analyzed by the BioMap (Novartis, Basel, Switzerland).

The phospholipid distribution obtained showed three patterns：1) cancer cell-specific localization, 2) stroma and stromal cell-specific localization, and 3) uniform distribution. Intriguingly, the distribution of PIs showed a unique localization. PI (18:0/20:4), one of the major molecular species of PI, was localized in stromal cells while it was almost undetectable in cancer cells. In contrast, PI (18：0/18：2), PI (18：1/16：0) and PI (16：0/16：0) were mostly observed in only the cancer cells. PI (18：0/22：6), PI (18：1/20：4), PI (16：0/20：4), and lysoPI (18：0/OH) were uniformly distributed while the signal intensities of the first three PI were dramatically lower than that of lysoPI (18：0/OH). PA (16：0/18：1), a precursor of PI, was detected in cancer cells. From these findings, we could speculate that this replacement of acyl-chain or de novo PI synthesis occurred in the breast cancer and regulate the PI 3 K or arachidonic acid pathway in the cancer cells, although we didn't compare with the normal ductal and stromal cells. At least, majority of PIs in cancer cells could be a palmitic acid or stearic acid containing PIs. In addition, The palmitic acid containing phosphatidylcholine (PC), PC (16：0/16：0) and unknown molecular species of PC, which were localized in cancer cells changed their sn-1 acyl-chain from palmitic acid (16：0) to palmitoleic acid (16：1) in accordance with the distance from the stromal region. Although this was observed only in one case, we thought it was because each tumor cell cluster was very small in another case.

In summary, we observed the unique localization of PIs and PCs in breast cancer tissue using high-resolution imaging mass spectrometry. We will confirm these findings by measuring the tissues containing normal ductal and stromal cells and investigate the key factors related to the PIs and PCs reconstruction.

PMo-083
11:10 – 12:20

Oxygen defect chemistry proposed by zinc and antimony images in the dense tin dioxide ceramics by means of NanoSIMS 50

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Keywords:
Transparent oxides, high-resolution imaging, defect, tin dioxide

Novel aspects:
We studied the oxygen defect chemistry through zinc and antimony distribution in tin dioxide ceramics.

Abstract:
Transparent oxides such as zinc oxide and tin dioxide, an n-type semiconductor with a wide band gap, has been extensively studied as a useful material for application to solar cells, gas sensors, and oxidation catalysis. It is considered that the origin of n-type conduction is oxygen vacancy. Especially, the study on oxygen defect in tin dioxide is not enough, because it is difficult to produce dense ceramics and single-crystal. In this study, we propose new defect chemistry on the oxygen vacancy through the distribution of zinc and antimony in the dense tin dioxide ceramics.

PMo-084 MALDI Imaging of Metabolites Reconstructed by CE-MS Based Quantitative Analysis

13:30 – 14:40

Tsuyoshi Nakanishi[1], Takako Hishiki[2,3], Shigeki Kajihara[1], Kiyoshi Ogawa[1], Makoto Suematsu[2,3]

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Keywords:
MALDI, imaging mass spectrometry, CE-MS, hypoxia

Novel aspects:
Evaluation of the distribution and the content of various metabolites by using metabolite maps reconstructed from the CE-MS based quantitation

Abstract:
Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry has been widely used to display the spatial localization of endogenous metabolites and administered pharmaceutical drugs in various tissues. This technique is currently used to the wide variety of animal and plant systems. In MALDI imaging mass spectrometry (IMS) technique, to keep a high sensitivity and a good reproducibility, a homogeneous matrix coating on tissue slices is required. As a typical matrix coating method, a spray method, a droplet method using a robotic spotter and a vapor deposition technique are known and each method is chosen according to the aim (a spatial resolution, the reproducibility, an extraction efficiency of molecules, a cost, etc.) . To keep the reproducibility between multiple samples on IMS experiment, we performed matrix deposition by usage of a robotic spotter. In this study, we performed IMS of endogenous metabolites in a mouse brain tissue using 9-aminoacridine as a MALDI-matrix. As a consequence of IMS, these molecular images displayed a unique distribution of each metabolite between multiple samples and these molecular images suggested homogenous matrix deposition between many spots led to acquire reproducible results among different slices.

Subsequently, to reflect the concentration of metabolites in tissues to the molecular images, the quantitative data by CE-MS was tried to couple to imaging of metabolites. Quantitative analysis by CE-MS was achieved using a sequential tissue slice next to a tissue slice for IMS measurement. First, a ratio of the peak intensity of a target metabolite at *n*th spot (Int_n) / the median of the peak intensities of the metabolite of the all the spots (Int_{ave}) was calculated from the IMS data and next, the apparent concentration (C_n) of each metabolite at the *n*th position of tissues was determined by multiplying the ratio by the metabolite concentration (C') of tissue quantified by CE-MS. Thus metabolite maps (AMP, ADP and ATP) were reconstructed to evaluate the changes of energy metabolism among the multiple samples. Furthermore, energy charge (EC) was also calculated using reconstructed molecular images from AMP, ADP and ATP. Using this IMS technique linked to the quantitation, we displayed the distribution of ATP and its degradation metabolites, ADP and AMP in mouse brain tissues from both wild type and transgenic mice deficient with a target enzyme under the normoxia/hypoxia. Molecular imaging reconstructed by CE-MS based quantitative data could become a powerful tool to evaluate the fluctuation of metabolites between multiple tissue slices from different mice, reproducibly and reliably.

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PMo-085
11:10 – 12:20

New nano ESI source development for increased performance nanoLC-MS with plug-and-spray configuration

Reiko Kiyonami[2], Peter Soendergaard[1], Christian Ravnsborg[1], Vlad Zabrouskov[2]

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Keywords:
nano ESI-source, column heater, column/emitter integration

Novel aspects:
Newly developed nano-electrospray source allows non-LC-MS experts to get optimized nanoLC-MS results by using an integrated column-emitter design.

Abstract:

Introduction

Nano-flow LC-MS is widely used for qualitative and quantitative proteomics studies due to its high sensitivity and specificity ; however, it is frequently observed that irreproducible and poor results are obtained owing to imperfect connections of tubing, column, high voltage electrode, and the emitter. Improper connections often result in leaks and large swept volumes that cause substantial peak broadening and thus poor sensitivity while a poor high voltage connection will yield poor data because of unstable spray. In order to address these common issues, we have developed a new nano-electrospray source and emitter concept in which, a column, column heater, high voltage electrode and an emitter are combined in one ready-made assembly. The performance of this new source concept was evaluated.

Methods

A prototype of new developed nano-electrospray source was installed on the Orbitrap Elite mass spectrometer and connected to an Easy-nLC 1000 nanoLC pump. Three column (50 µm id x 15 cm, 2 µm) assemblies were used for evaluating column-to-column and run-to-run reproducibility. Both complex peptide mixtures and simple digest mixture were used. The retention time reproducibility, peak shape, resolution and peak capacity in different temperature ranges were evaluated. In order to test the device flexibility, several flow rates from 150 nl/min to 1000 nl/min were used. The mobile phases were 0.1% FA/H 2 O and 0.1% FA/MeCN. A 60 min linear gradient was used for the complex sample separation. A 15 min linear gradient was used for the simple digest sample separation

Preliminary Data

The column assembly is positioned in the source without the need to adjust the X,Y, or Z positions of the emitter. A high spray stability was achieved over all LC runs with three different columns. The obtained chromatographic resolution, sensitivity, and reproducibility matches other state-of-the-art data without need for adjustments (or expert intervention) beyond plugging in the column/sprayer assembly. More than a thousand proteins were identified with increased component detection from the complex protein digest mixtures. The reproducibility of the retention time from column to column for the targeted peptides from the simple standard mixtures was less than one percent.

PMo-086
13:30 – 14:40

Electrostatic Spray Ionization and Microchips Emitter for On-Line MS Monitoring of Chemical and Electrochemical Reactions

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Keywords:
Electrostatic Spray Ionization, Microfluidic chip, Mass Spectrometry, Nitration

Novel aspects:
The ESTASI provides an alternative strategy to ionize a sample from a surface as compared to laser desorption ionization and desorption electrospray ionization.

Abstract:
An electrostatic spray ionization (ESTASI) method has been developed for mass spectrometry (MS) analysis of samples in or on an insulating substrate.[1] The principle is based on the capacitive coupling between an electrode and the sample. In practice, a metallic electrode is placed close to the sample and isolated by an insulator. Upon application of a high voltage pulse to the electrode, an electrostatic charging of the sample occurs leading to a bipolar spray pulse. When the voltage is positive, the bipolar spray pulse consists first of cations and then of anions. This method has been applied to a wide range of geometries to emit ions from samples in a microchip, in a silica capillary, in a disposable pipette tip or from samples on a polymer plate.

Specifically, proteins or peptides separated by an isoelectric focusing gel electrophoresis can be directly profiled by the ESTASI-MS without staining and extraction procedures. Indeed, with the ESTASI-MS, proteins with an amount of several nanograms can already be detected, indicating a strategy more sensitive than the classical Coomassie Brilliant Blue staining and providing the molecular weight or even fragmentation information at the same time. This protein profiling strategy with ESTASI-MS has a brilliant future in top-down proteomics.

We have also developed multi-functional polymer microchips as both microreactors and electrospray emitters for on-line analysis of reaction products by MS. Typically, the microchips include several microchannels to introduce reagents to a main reaction channel, electrodes to apply high voltage for electrospray or apply voltages for on-line electrochemical reactions, and a microchannel to provide sheath flow to stabilizing electrospray ionization (ESI) . When the electrode providing high voltage is in contact with the main channel for spray, the microchip is used for regular ESI. When the electrode for high voltage is isolated from the main channel by polymer, the microchip is used for capacitive ESTASI.

In a first application, the microchip-MS was used to investigate the nitration of tyrosine.[2] With the advantages of on-line reaction, we have found new mechanisms for nitration on tyrosine catalyzed by copper (II) . In general, micromolar concentrations of copper catalyze the nitration process by nitric oxide (NO) oxidation, whilst millimolar concentrations catalyze the nitration process by nitrite oxidation. Comparing to batch reaction, the on-line microchip-MS system is useful to detect reaction products with short lifetime produced in seconds, such as radicals. When different solvents are introduced via different microchannels, the microreactor can be used for the research of liquid-liquid interface reactions. We have investigated the mechanism of ferrocene methanol oxidation at the 1,2-dichlorobenzene-water interface.

Since sheath flow of a buffer suitable for ESI is provided, the microchip can be used as an emitter to connect with various microfluidic devices for efficient and stable ESI of analytes. In one example, we have connected the microchip emitter with a push-pull probe developed in our group for scanning electrochemical microscopy (SECM) .[3] Therefore, surface chemical component was firstly characterized and imaged by SECM and then extracted for microchip-ESI-MS imaging. In another example, the ESI emitter microchip was connected with a microfluidic chip for electrochemical reaction, similar as the one reported recently in BIOSENSORS&BIOELECTRONICS.[4] Drug metabolites generated by electro-oxidation was analyzed on-line by ESI-MS.

Reference
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PMo-087
11:10 – 12:20

Miniaturized Peptide Sample Pretreatment Platform for Indirect Spray Analysis

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Keywords:
indirect spray, solid phase extraction, sample pretreatment, ambient ionization, peptides

Novel aspects:
Miniaturized peptide sample pretreatment platform that only required nano-liters of sample. Subsequent indirect spray mass spectrometric analysis can be carried out using same platform.

Abstract:
The presence of salt or matrix components is known to suppress analyte signal[1] in the mass spectrometric analysis of complex mixtures. Therefore, solid phase extraction (SPE) is an important and widely used sample pretreatment technique[2] to preconcentrate the target analyte and remove interfering components prior to the mass spectrometric analysis. However, the minimal volume of sample required for SPE clean-up is typically one to a few micro-liters.
Our group has developed a sensitive ambient ionization technique, named indirect spray[3]. Using membrane funnel, 1 fmol of peptide is sufficient to provide detectable signal in a Fourier-Transform Ion Cyclotron Resonance mass spectrometer. Sample volume requirement for an indirect spray analysis can be as low as 50 nL. To couple the advantages of SPE and indirect spray, SPE sorbent attached membrane funnel is prepared. Preliminary results showed that SPE in sorbent attached membrane funnel format was feasible. Angioteinsin II could be adsorbed on the C18 sorbent efficiently by simply loading the sample onto the surface of the sorbent film. After the washing step, angiotensin II signal could remain essentially the same as before washing. Desalting was feasible for peptide sample prepared under physiological concentration of salt (150 mM NaCl) . Determination of salt tolerance, reproducibility and sensitivity of indirect spray using this miniaturized peptide sample pretreatment platform are underway. We will also explore the applicability of other sorbent materials for specific peptide analysis.

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PMo-088 Novel off-line electrospray ion source with a disposable needle

13:30 – 14:40

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Keywords:
ElectrosprayIonization Off-lineESI DisposableNeedle

Novel aspects:
Novel off-line electrospray ion source with a disposable needle for liquid sample analysis.

Abstract:

Introduction

Electrospray ionization is a useful tool for analyzing liquid samples, such as environmental, pharmaceutical, and biological samples. Usually, a liquid sample is pumped through a metal capillary of the electrospray ion source. The capillary is contaminated and then is sometimes clogged by dirt that is in the sample.

Off-line ion sources such as probe electrospray[1] and ESI chip[2] were reported to reduce contamination and blockages. For further improvement, we have developed a novel off-line electrospray ion source (off-line ESI) using a disposable needle that is available commercially and is easy to exchange. We will show the preliminarily results obtained using an off-line ESI with a disposable needle.

Methods

Our off-line ESI consists of a disposable needle. The disposable needle consists of a metal capillary and a plastic reservoir with a volume of about 100 uL. A liquid sample was introduced from the end of the metal needle into the sample reservoir using a syringe that was connected to the reservoir. After supplying the sample, the syringe was separated and a high voltage of 3 - 5 kV was applied to the metal needle. The liquid sample was continuously sprayed from the end of the disposable needle by gravity (without pumping) .

Preliminary data

Reserpine was used as a measurement sample, and the following three solvents were used : methanol, methanol/water : 1 /1, and water. The ion sensitivity of reserpine ions using the off-line ESI with the disposable needle was about half that of the normal ESI. The signal lasted for over 60 seconds with an inner diameter of 0.19 mm.

We investigated the flow rate by gravity dependence on the inner diameter of the capillary for several solvents. For methanol, the flow rates with an inner diameter of 0.13, 0.19, 0.26, and 0.33 mm were 20, 50, 200, and 600 uL/min, respectively. The flow rate increased proportionally to the 3.7th power of the capillary's inner diameter, which is consistent with the conductance of a capillary being proportional to the 4 th power of the capillary's inner diameter. The flow rate of the methanol /water : 1 / 1 was reduced to 40-50 % of that of the methanol solvent, and the flow rate of the pure water was reduced to 70-80 % of that of the methanol solvent. This result shows that the flow rate is approximately inversely proportional to the viscosity of the solvent.

References

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PMo-089
11:10 – 12:20

Movement of Ions and Charged Droplets from Atmospheric Region into the Gas Dynamic Interface

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Keywords:
Transport capillary, gas-dynamic Interface, ESI, APCI

Novel aspects:
direct separate measurement of ion and droplet losses in their movement through a transportation capillary from atmospheric region into the interface

Abstract:
Mass-spectrometry with ambient ionizations requires ion transfer into the high vacuum region. A nozzle or transportation capillary is often used in modern instruments ; a number of vacuum steps are used before an ion enters the high vacuum. Physical processes in ion movement from the atmospheric region to the gas dynamic interface, i.e.the low vacuum part of the instrument, are very important as they affect strongly on main analytical features : the mass range, detection limit, and precision.
The transport capillary is a cylindrical channel with the diameter D and length L. The nozzle can be treated as a capillary with extremely small length. Droplets can be evaporated in their movement through the capillary, and the charged particles are able to diffuse to its walls ; this results in loss of signal and instrument discriminations.
The presented work discusses dependence of the capillary transmission on the L/D relation, of a sort of charged particles, and a magnitude of the through the capillary. Capillaries have been used with L/D ratios of 0.8, 32.5, and 420, and lengths L0.3, 30, and 150 mm. Studies were carried out by a double focusing mass spectrometer MX-3303 with a Kontorovitz-Grey gas-dynamic interface in the positive ion mode.
Atmosphere pressure corona ionization (APCI) and electrospray ionization (ESI) have been used to get various types of the charged particles : APCI generates only ions ; they are mostly water clusters and provide a steady and high ion current. On the contrary, ESI gives both ions and charged droplets, and their relative role in the total current depends on the spraying details.
It was shown experimentally that the capillary transmission decreases with the increase in its relative length. In the APCI mode of ionization with the input current of 1500 nA, the current transmission drops by a factor of three when the capillary with L/D =0.8 has been substituted by the one with L/D = 32.5 ; for the capillary with L/D = 420, the transmission was decreased by a factor of 50. In the ESI mode, the input current was ~ 60 nA, and the drop in transmission was 3 times for L/D =32.5, and ~150 times for L/D = 420.
To determine the charged particle type entering the gas dynamic interface, we applied retarding potential to the skimmer : the electric and gas dynamic forces work oppositely. The voltage of 20- 80 V was enough to repulse ions as they have rather high mobility ; however, it was too low to affect droplets. This made it possible to evaluate the efficiency of droplet evaporation inside the transportation capillary. To validate the above ideas, detailed simulation of ion and droplet movement have been done using SIMION 8.0 and ASXIM.
The output current consisted of droplets for more than 99% for a nozzle or short capillary ; as for the capillary with L/D = 420, the 30 % of output current were ions. This means that droplets are evaporated actively in the capillary, and this effect can not be ignored when one estimates the relative effectiveness of different methods of introducing the charged particles into a gas-dynamic interface. We have simulated the gas dynamic movement inside all three types of the capillary using Fluent 6.0 and calculated the charged particle diffusion to the capillary wall. The results are in good agreement with the experimental findings.

PMo-090
13:30 – 14:40

An introduction of a novel atmospheric pressure Micro/Nano-ESI ion source

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Keywords:
Electrospray, Sources, Micro/Nano-ESI

Novel aspects:
A novel micro/nano-ESI ion source provides ease of use, improved ion signal stability, and better S/N ratio.

Abstract:

Introduction

The advent of micro/nano-ESI has considerably extended the applications of ESI in analytical mass spectrometry. It offers some remarkable benefits such as lower sample consumption and higher ionization efficiency. However, conventional micro/nano-ESI systems can suffer from unstable Taylor cone effects, clogging of emitters, and incomplete droplet desolvation, making them more difficult to operate. In the present study, a novel source was developed to accommodate nano to micro LC flow ranges and operate atatmospheric pressure. Compared to the conventional micro/nano-ESI ion source, this new ion source offers ease of use, 2 fold improvement in signal stability, and higher S/N ratio with more complete droplet desolvation.

Methods

The experiments were carried out on an IONICS EP10+ with IONICS HSID™ interface. Comparisons of signal stability, reproducibility, and S/N ratios were made for the new ion source and a conventional microspray source. Flat-cut silica capillaries : 25 um (ID) were used as sprayer emitters. Reserpine (100pg/uL) in H_2O : MeOH (1 : 1) with 0.1% acetic acid was used to study the signal stability with both syringe and nano-HPLC pumps. Both BSA (tryptic digest) (50fm/uL) in H_2O (100%) with 0.1% formic acid and testosterone (10pg/uL) in serum were run with nano LC columns (75um (ID) , C-18) to study the S/N ratio of the MRM spectra. The LC gradient was 5 % to 95%in the organic solvent in a 15-minute run.

Preliminary data

The ion source conditions such as sprayer emitter position, gas flow, and sprayer voltage, were fully optimized. For this new ion source, the optimization process was simple and straightforward with good reproducibility, offering ease of use. However, for the conventional microspray ion source, the optimization process was very time consuming with poor reproducibility. There were also larger ion signal variations at different ion source conditions for the conventional microspray source. After the ion source optimization, the ion signal stability was measured with a reserpine sample using both syringe and nano-HPLC pumps.

The variation in the total ion counts from Q1 scans were recorded. The results consistently showed that the new ion source had 2-fold improvement in stability compared to the microspray ion source for both syringe and nano-HPLC pumps. Using a nano-HPLC pump with 75umID columns, the MRM spectrum from multiple injections of reserpine also showed that the new ion source provided two times higher signal reproducibility than that of the microspray ion source. The MRM spectra from a BSA digest were examined to compare the S/N ratio for both ion sources. The results clearly showed reductions of chemical noise level of the new ion source due to the more complete droplet desolvation. Although the absolute ion intensity of the microspray ion source was better than that of the new ion source, the resulting S/N ratio of the new ion source was better. A similar trend was also found in the MRM spectra of testosterone in serum. Since the MRM spectrum itself can greatly reduce the chemical noise level, the Q1 multiple ion (MI) scan of a BSA digest was also used to examine the background noise and S/N ratios. The result clearly showed improved S/N ratio with the new ion source.

PMo-091
11:10 – 12:20

Electrospraying, Movement and Evaporation of Charged Droplets Using Rotating Gas Flows and Transverse Ion Extraction

Sergey Masyukevich[1], Nikolai Gall[2], Natalia Fomina[1], Mihail Lapushkin[2], Alexandra Kretinina[1]

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Keywords:
Electrospraying, Rotating Gas Flows

Novel aspects:
new ESI design with rotating gas flow and transversal ion extraction

Abstract:
The mass-spectrometer sensitivity are highly determined by droplet eletrospraying, their movement by gas and electric forces, evaporation, and ion collection to the sampler. The most significant signal loss occurs in the ambient region because of spray inefficiency, imperfect droplet evaporation and spatial repulsion of the charged particles. The signal losses due to each of these reasons can be of 2 to 3 orders of magnitude : the total loss in signal compared to its maximal theoretical value is often as high as 10^5 even for effective commercial instruments.
We have examined the sequence of physical processes that leads to losses in ions at the atmospheric region, and indicated the mutual relationship between the named mechanisms. Generally, electrospray proceeds under the influence two electric fields : the outer electric field created by the counter electrode and the space charge field created by droplets emitted from the Taylor cone. Space charge field plays an important role, suppressing the spraying at high flow rates, and causing high and low frequency oscillations of the emitted current.
Evaporation of droplets is limited by the drift time of their movement, by heat flux, and significant readsorbing of solvent molecules. Droplet move much more slowly than ions, and are the main source of space charge to cause an effective repulsion of the particles.
We have studied experimentally, in simulation and theoretically the possibility of electrostatic focusing of ions at the atmospheric pressure by electrostatic lens system. It was shown that the nature of particles and the space charge density are the main factors determining focusing efficiency. Thus for ions got from corona ionization, the focusing efficiency dropped from ~ 15% at 50 nA down to 0.1% at 1 mA. Focusing almost did not work at the first situation : the current part reaching the sampler entrance was nearly the same as ratio the geometric orifice area and the area of the ion cone. In the case of ESI, the situation was much more complicated and depended on the temperature and solvent composition
To overcome these difficulties, we have developed a novel spray system, based on a combination of droplet evaporation in a rotating heated gas and electrostatic ion extraction in the transverse direction. The measurements showed a significant increase in sensitivity of the instrument, as high as two orders of magnitude for favorable conditions, and sufficient increase in spraying effectiveness for high sample flow rates

PMo-092
13:30 – 14:40

A reference method for the SI-traceable quantification of somatropin in human serum using an isotopically labeled protein internal standard

Caroline E Pritchard[1], Milena Quaglia[2], Alison E Ashcroft[3], Gavin O'Connor[2]

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Keywords:
protein quantification, SI-traceable, IDMS

Novel aspects:
Use of isotopically labelled protein internal standards to perform SI-traceable quantification in human serum samples.

Abstract:
The need for certified reference materials is well recognized within the clinical community and by the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) . Use of suitable reference materials supports results that are accurate, specific, and most importantly comparable between laboratories. Although methods for the traceable quantification of standard protein solutions have now been developed, such standards are rarely commutable for use with routine clinical or biochemical immunochemical measurement techniques. Solution standards behave differently during the analytical process than the complex biological samples they are being used to standardise. Consequently, there is a need to develop methods that give traceable results for protein standard materials in complex matrices such as human serum.

Using somatropin (recombinant human growth hormone, rhGH) as a model, a method for the SI-traceable quantification of a specified protein inhuman serum has been developed. Isotopically labelled rhGH was used as an internal standard to perform isotope dilution mass spectrometry (IDMS) . This should account for any losses encountered during sample preparation, incomplete digestion due to the presence of the remaining matrix proteins and any modifications that occur during the method. In order to remove any structural differences between the natural rhGH and its labelled analogue, denaturing and reducing conditions were used prior to sample clean-up. Removal of the large (>30 kDa) proteins within serum and subsequent reverse-phase fractionation was performed using a restricted-access type media. The resultant simplified matrix was digested using trypsin and quantification of the tryptic peptides released was performed by mass spectrometry.

In order to achieve traceability, a rhGH solution standard previously quantified using internal labelled peptide standards was used to spike blank human serum. A serum sample was prepared at 10 ng/g and quantified using the method described to give results traceable to the SI with a total measurement uncertainty of <20 %.

PMo-093 Large-scale LC-MS-based Profiling of Soluble Protein Complexes

11:10 – 12:20

Cuihong WAN[1], Pierre C Havugimana[1], Andrew Lugowski[1], Kathy Foltz[2], Andrew Emili[1]

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Keywords:
protein-protein-interactions ; LC-MS/MS ; HPLC ; co-elution ; seaurchin

Novel aspects:
Global examination of protein complexes in sea urchin development using a novel high-through screening approach based on high-performance HPLC separation and LC-MS/MS analysis

Abstract:
Macromolecular complexes are essential for all aspects of cell biology, including development. Generating comprehensive cellular protein-protein interactions maps is therefor eessential for understanding the basic molecular biology of organisms. While progress has been made in the large-scale isolation and identification of stable protein complexes simple model organisms like yeast, existing high-throughput assays for mapping physical interactions like affinity purification are difficult to apply to non-traditional classical model organisms such as sea urchin. Here we describe the application of an integrative proteomic profiling approach for systematically enriching and identifying the components of soluble protein complexes during early stages of development in *Strongylocentrotus purpuratus*. Soluble protein was extracted from lysates of eggs prior to fertilization, 5 min post-fertilization, at 2-cell cleavage stage, and from hatched blastulas, and subjected to extensive non-denaturing fractionation with mix-bed ion-exchange high pressure liquid chromatography (IEX-HPLC) . Proteins belonging to the same complex that co-elute during HPLC are then identified and quantified by tandem mass spectrometry (LC-MS/MS) using a high precision Orbitrap-velos instrument. SEQUEST and PepQuant (homemade software) were used for MS/MS database searching and label-free MS 1 precursor ion-based quantification, respectively. Interaction scores were assigned based on the similarity in fractionation profiles (ie Pearson correlation score) of co-eluting protein pairs based on the recorded spectral counts and peak intensities. Next, an integrative machine-learning procedure using supporting biological evidence (e.g. co-evolution, functional annotations, co-expression, co-localization, conservation) was used to filter the protein protein interactions to generate a high-confidence PPInetwork, while clustering was applied to predict protein complexes. This work provides an unprecedented perspective into the global molecular organization and conservation of protein complexes in sea urchin cells during early development, and should facilitate further mechanistic insights into animal gene function.

PMo-094
13:30 – 14:40

Mass spectrometry-based sequencing of protein C-terminal peptide using alpha-carboxyl group specific derivatization and COOH capturing

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Keywords:
C-terminal sequencing of protein

Novel aspects:
The C-terminal enrichment was successfully performed by double digestion using GluC and AspN, and COOH selective capture with tosylhydrazide glass material.

Abstract:
The C-terminus of protein is of great importance in determining various cellular functions. Presently, mass spectrometry (MS) has been the method of choice for analyzing the protein C-terminus, but even with the cutting-edge technology, it is still difficult to analyze the C-terminus of a protein of interest, and it is not too much to say that there are only a few methods for the analysis. The conventional methods of peptide mass fingerprinting and peptide fragment fingerprinting can often not give any information of C-terminal structure, in particular when the protein is C-terminally processed or posttranslationally modified. Hence, an efficient and practical technique for characterizing protein C-terminal variation has strongly been needed.
We here present an approach to MS-based sequence analysis of selectively enriched C-terminal peptide from protein. This approach employs a combination of the specific derivatization of alpha-carboxyl group (COOH) , proteolytic double digestion using endoproteinase GluC and AspN, and enrichment of C-terminal peptide through the use of COOH-capturing material. Highly selective derivatization of alpha-COOH was achieved by a combination of specific activation of alpha-COOH through oxazolone chemistry and amidation using 3-aminopropyltris (2,4,6-trimethoxyphenyl) phosphonium bromide (TMPP-propylamine) . This amine component was used to simplify fragmentation in MS/MS measurement, which facilitated sequence determination. The peptides produced after GluC/AspN digestion were then treated with a COOH scavenger to enrich the C-terminal peptide that lacks COOH group, and the obtained C-terminal peptide was readily sequenced by MALDI-MS/MS due to the TMPP mass tag.
In the current status, there is no general method for C-terminal sequencing analysis of protein, partly due to its highly varied nature, hence versatile techniques for such purposes have been required. The method presented in this session can be used complementarily with previously reported protocols for protein C-terminal analysis.

PMo-095
11:10 – 12:20

Identification of proteins with different expression profiles related to Nasal Polyps in Aspirin Intorelant Asthma(AIA) using MALDI-TOF/MS

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Keywords:
Proteomics analysis,MALDI-TOF/MS,Nasal polyps,Aspirin Intolerant Asthma (AIA)

Novel aspects:
Proteomics analyasis is one of usefull methods for investigate mechanisms of AIA.

Abstract:
Introduction: The mechanism of aspirin intolerant asthma (AIA) remains unclear. AIA patients have a character with intractable nasal polyps. We performed proteomics analysis of nasal polyps to identify proteins related to AIA using different expression profiles.

Methods: Proteomics analysis by nasal polyps had been performed in AIA and Chronic Rhinosinusitis (CRS) . Polyp samples were taken from 8 patients with AIA and 8 patients with CRS. Proteins extracted from polyps were labeled with Cy 3 or Cy5. Samples were run 2 D-DIGE (2 dimensional differential gel electrophoresis) . The target protein spots were found with Progenesis PG240 software. And then these were subjected MALDI-TOF/MS analysis. The database search for peptide mass fingerprinting candidates was performed using a Mascot Search Program. Immunohistochemistry and western blotting by using antibodies against these proteins were followed in nasal polyps for AIA and CRS. Finally, in vitro, we studied regulation of these proteins using EoL- 1 which is human eosinophilic cell lines.

Results and Discussion : We identified 122 diffrentially expressed protein by 2 D-DIGE. Compared with CRS, there were 79 proteins up-regulated and 43 proteins down-regulated. Among these, 4 proteins including protein X (temporary name) were highly overproduced in AIA patient's nasal polyps. To validate the results obtained in this study, we selected one candidate protein (protein-X) related to AIA and assesed more detail. The differential expression of protein-X in cytoplasm of eosinophil was confirmed by immunohistochemical analysis. Aspirin and lipopolysaccharide (LPS) has not changed protein-X mRNA in EoL-1. CysLT 1 R mRNA was up-regulated by LPS and decreased by transfected siRNA for protein-X.
Proteomics analyasis is one of usefull methods for investigate mechanisms of AIA. Ptrotein-X is overexprresed in AIA patient's nasal polyps and have important role of AIA as LPS.

PMo-096
13:30 – 14:40

Specific Racemization of a Cysteine Residue in the Hinge Region of Immunoglobulin Gamma 1 during Storage

Masato Amano[1], Naoki Kobayashi[1], Takashi Nakazawa[2], Susumu Uchiyama[3], Kiichi Fukui[3]

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Keywords:
Antibody Therapeutics, Hydrogen/Deuterium Exchange, Racemization

Novel aspects:
Finding of racemization in antibody therapeutics and investigation of analytical method for racemization in protein with hydrogen/deuterium exchange mass spectrometry.

Abstract:
Therapeutic antibodies often suffer from degradation due to various modifications during storage. We detected a novel degradation of immunoglobulin gamma 1 (IgG1) stored for 6 month at 40 degrees celsius, and identified the modification as the racemization of a cystein residue in the hinge sequence of heavy chain. The racemization was confirmed by mass spectrometry in conjunction with extracted ion chromatography of the tryptic digest of IgG1 forced to degrade in D_2O. To rationalize the possible relationship between the racemization of cysteine, we suggest a new reaction mechanism that assumes a base catalyst to initiate these reactions by activating the amide nitrogen of neighboring lysine residue.

PMo-097
11:10 – 12:20

Identification and affinity characterization of carbohydrate-binding epitopes in human Galectin-9 and rat Galectin-5 by proteolytic Excision-MS and bioaffinity analysis

Frederike Eggers[1], Adrian Moise[1], Sabine Andre[2], Hans-Joachim Gabius[2], Michael Przybylski[1]

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Keywords:
CREDEX-MS, CRD, SAW-bioaffinity, Affinity-MS, SAW-ESI-Ion-Trap-MS-online-coupling-system

Novel aspects:
SAW-bioaffinity analysis of carbohydrate peptide/protein interactions.New clinically relevant carbohydrate binding epitopes.

Abstract:
The emerging physiological significance of carbohydrate (glycan) -protein (lectin) interactions direct increasing attention to the structural analysis of this contact (1). Using human adhesion/growth-regulatory galectins, we have developed a robust and reliable approach to identify sequence stretches in contact to the ligand. In this study we want to focus on Galectin-9 and Galectin-5 because sequence homology analysis revealed that the C-terminal carbohydrate-binding domain of galectin-9 has extensive similarity to that of monomeric galectin-5. In contrast to galectin-5, which is mainly expressed in erythrocytes, galectin-9 was found to be widely distributed, *i.e.* in liver, small intestine, thymus, kidney, spleen, lung, cardiac and skeletal muscle, reticulocyte and brain. Galectin-9 is also known to be a selective eosinophil chemoattractant and it was found in tumor tissue of Hodgkin's disease. Because of such features characterisation of galectin-carbohydrate interactions and identification of binding sites is of crucial importance for drug design. Combination of proteolytic excision and affinity-mass spectrometry yielded peptides reactive with cognate sugars, as ascertained by biosensor and cell assays.

Experimentally, galectins were adsorbed to an affinity resin bearing cognate sugar and proteolytically digested. After washing out unbound galectin fragments, the fragments remaining on the column were eluted, and both fractions analyzed by mass spectrometry. This procedure was termed CREDEX-MS (Carbohydrate recognition domain excision/extraction). The identified CRD-peptides were synthesized by Fmoc-SPPS and their binding properties characterized by affinity-MS. The interactions of lactose with full-length galectins and with identified CRD-peptides were studied by SAW-bioaffinity-measurements and online-bioaffinity-coupling of SAW with ESI-MS. The lactose was immobilized on bioaffinity-sensor-chips in the form of a glycopeptide. SAW measurements were performed with an S-Sens K5 Biosensor instrument in PBS buffer and elution was performed with ACN : 0.1% TFA 2 : 1.

CREDEX-MS of galectins identified at least two carbohydrate-binding peptides for every galectin - in complete agreement with the binding sites of the crystal - or molecular modelling structures. All identified carbohydrate-binding peptides were synthesized and their affinity for lactose demonstrated by affinity-MS. To get more detailed information about the affinity and interaction kinetics of the CRD peptides and full galectins with lactose, SAW-bioaffinity measurements were performed. Lactosyl-glycoprobe was immobilized on the SAW chip and dissociation constants of galectin peptides and galectins with lactose determined to be in the µM range. For a real-time study of carbohydrate-lectin interaction a newly developed online-coupling of SAW with ESI-Ion Trap MS was used. With this system the peptides interacting with on chip bound carbohydrate could be studied directly after the elution and it could be proved which peptides interact with the carbohydrate on the chip. This system was tested with synthetic CRD peptides and it was shown that online-coupling of mass spectrometry with the biosensor was successful.
The presented results document the validity of the concept to obtain bioactive peptides from lectins (2). These bioinspired peptides are becoming objects of detailed structural analysis and inspire their tailoring, e.g. by incorporating non-natural amino acids, as medically useful mini-lectins or lectin blockers.
Surface acoustic wave measurements were shown as a working tool for determination of dissociation constants as well as in combination with ESI-Ion-Trap-MS for the study of carbohydrate-lectin-interactions.

PMo-098
13:30 – 14:40

Differentiation of isobaric residues in SPITC-derivatized tryptic peptides using MS/MS technique in a novel Curved Field Reflectron.

yuzo yamazaki, Keisuke Shima

Shimadzu Corporation, Kyoto, Japan

Keywords:
MALDI, CID, SPITC, isoform, CFR

Novel aspects:
Specific fragment ions obtained by a novel CFR can discriminate isobaric residues in SPITC-derivatized peptides without impairing interpretable sequence information.

Abstract:
Fixing a strong negative charge at N-terminus of tryptic peptide is a quite effective chemical derivertization for *de novo* sequencing by using post-source decay (PSD) on MALDI-TOFMS. However, whereas the chemical derivertization causes interpretable y-ions mainly, one cannot differentiate isobaric amino acid residues, for instance, Ile/Leu and α- / βAsp. We will report our study of differentiation of these residues by using PSD and a high energy CID in a novel curved field reflectron.
PSD and high-energy CID-MS/MS in the curved field reflectron were performed in positive ion mode. For the high-energy CID experiments, helium was used as collision gas, and a collision energy was 20 keV (laboratory frame of reference) . 4-sulphopenyl isothiocyanate (SPITC) was purchased from SIGMA. Tryptic digests of BSA and β-casein were provided to SPITC-derivatization, as described in the previous report. Some peptides including Ile/Leu were analyzed by PSD and the MS/MS. Two synthesized peptides, which include α- and β Asp respectively, were also provided to the derivatization, and then analyzed by MS as well.
As reportedly, the derivertization of the tryptic peptides produced y-ions mainly in PSD measurement, which is useful to interpret sequences *denovo* easily. However, it was impossible to differentiate Ile and Leu due to the same residual mass. The high-energy CID-MS/MS (HE-CID) of the derivertized peptide produced moderate intensities of w- and d-ions that were generated by side-chain fragmentation, while dominant y-ions were still observed in the MS/MS spectra. It is noted that, in an operation of CFR, one can switch PSD to HE-CID so rapidly that it is easy to recognize d- and w-ions derived from Ile/Leu by comparison with both spectra. For instance, y-59 between two y-ions, a mass difference of which is 113, indicates Leu, while y-45 does Ile. Namely, by using HE-CID of the SPITC-derivertized peptide, it is easy to read an exact sequence from MS/MS spectrum, even though if the sequence includes Ile/Leu.
We also applied the derivertization to a differentiation of another isobaric residue, α- and βAsp. As we reported previously, PSD is applicable to differentiate Asp isomerization, because the isomerization causes significant changes in the intensities of fragment ions, which are responsible for cleavages at the N- and C-terminal sides of the isomeric residues. The SPITC derivatiztion induced more cleavable N-terminal side of the residue than the one in a underivertized counterpart. Notably, the specific y-ion ratios of Asp isomers were still retained after the derivertization, which is thought to be useful to perform further quantitative analysis.

PMo-099
11:10 – 12:20

Study of hydrogen-induced cleavage of peptides with MALDI-ISD and sonolytic hydrolysis

Motoshi Sakakura, Mitsuo Takayama

Yokohama City University, Yokohama, Japan

Keywords:
In-source decay, MALDI, Hydrogen radical

Novel aspects:
Hydrogen-induced cleavage of peptides with MALDI-ISD and sonolytic hydrolysis methods has been compared from the stand point of the mechanism for both methods.

Abstract:
Introduction
Simple and efficient protein cleavage holds the key for the success of bottom up proteome analysis approach using mass spectrometry. In-source decay (ISD) coupled with MALDI has been recognized as efficient ladder sequencing method. MALDI-ISD is caused by hydrogen transfer from matrix to analyte, and can be successfully applied for sequencing protein and peptide. Here we report an additional method for sequence analysis of peptides. The method is consists of an ultrasound sonolytic device and reductive hydrogen donors such as 1,4-benzenedithiol. The sequence informations of peptides obtained with MALDI-ISD and sonolytic hydrolysis were compared on the standpoint of the hydrogen-induced reactions.

Experimental
Mass spectra were acquired on an AXIMA-CFR MALDI time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan). The sonication was performed with a Branson 450 sonifier (Danbury, CT, USA). The frequency and power used were 20 kHz and 50 W, respectively. Analytes used were horse myoglobin, glucagon, ACTH18-39, and phosphorylated peptide. Analytes were prepared as an aqueous solution at 10pmol/ul. The ultrasound was applied to 1000ul of the aqueous solution with addition of hydrogen donors such as 1,4-benzeneditiol. 100umol additives were added into 1000ul of the analyte solutions. Peptide solution was premixed in 1 : 1 ratio with serine doped CHCA ; then a volume of 1 uL of sample solution was deposited onto a MALDI sample plate and solvents removed by allowing evaporation in air room temperature.

Results and discussion
MALDI-ISD, which uses hydrogen transfer reaction, resulted in the formation of N-terminal side c-series ions of both peptide and protein analytes without loss or degradation of amino acid side chains and phosphate group. On the other hand, ultrasound sonolysis combined with hydrogen donors in aqueous solution was applied to cleave of peptide and proteins, and resulting products were analyzed by MALDI-TOF MS. The hydrolytic characteristics of peptides were that Xxx-Pro bond was more susceptible to the sonolysis than other amino acid residues, while the information originated from Xxx-Pro degradation never be observed in MALDI-ISD, and that hydrolysis occurred without any side chain degradation. MALDI mass spectrometric analysis of the products obtained with sonolytic hydrolysis gave information about amino acid sequence of the peptides without any byproducts. The mechanism for the sonolytic hydrolysis of peptides would be presented on basis of the acid hydrolysis, while the mechanism of the MALDI-ISD has been known as a laser-induced hydrogen attachment reaction to the carbonyl oxygen on the peptide backbone.
The characteristics of chemical acid hydrolysis of peptides in solution have been reported that the peptide bonds at the C-terminal side (Asp-Xxx), the N-terminal side of serine and threonine (Xxx-Ser/Thr), and both sides of glycine (Xxx-Gly and Gly-Xxx) are relatively sensitive to acid hydrolysis. Moreover, the acid hydrolysis of peptides often leads to the formation of dehydration products as a result of side reactions. Furthermore, the peptide bond at the N-terminal side of proline (Pro-Xxx) is insensitive to acid hydrolysis. From the degradation characteristics of peptides with sonolytic hydrolysis differing from chemical acid hydrolysis, it was suggested that the sonolytic may not be arisen from protons, but hydrogen radicals.
Consequently, although the degradation characteristics of peptides with sonolytic hydrolysis quite differ from both the MALDI-ISD characteristics of which the peptide bond at Xxx-Pro is insensitive to MALDI-ISD and the chemical acid hydrolysis, the use of both methods of MALDI-ISD and sonolytic hydrolysis was very useful for amino acid sequencing of peptides.

PMo-100
13:30 – 14:40

Electrochemically Assisted Reduction of Disulfide Bonds of Peptides and Proteins With On-Line Mass Spectrometric Detection

Martin Eysberg, Agnieszka Kraj, Hendrik-Jan Brouwer, Nico Reinhoud, Jean-Pierre Chervet

Antec, Zoeterwoude, the Netherlands

Keywords:
disulfide bond reduction, electrochemistry

Novel aspects:
Efficient reduction of disulfide bonds using a novel electrochemical approach based on a new electrode type and square wave pulses

Abstract:
Disulfide bonds are one of the most important post-translational modifications of proteins. They are stabilizing protein's 3-dimensional structure and are crucial for their biological function.
The reduction of intra- and intermolecular disulfide bonds is necessary for successful characterization and assignment of the bonding sites by MS.
Off-line reduction is performed using highly concentrated chemical agent (*e.g.* dithiothreitol (DTT)) that needs to be removed prior LC/MS analysis. Alternatively, thiol - free reducing agents as TCEP (tris (2-carboxyethyl) phosphine) can be used. However, the sample preparation remains laborious and difficult to combine with on-line LC/MS.
Moreover, the possibility of on-line disulfide bond reduction can be beneficial for the determination of disulfide bond arrangements or top down proteomics strategy, which relies on fragmentation of intact proteins without enzymatic digestion.

In this poster, we present electrochemically (EC) assisted reduction of biologically active peptides and proteins containing disulfide bonds followed by on-line mass spectrometric detection. For the first time the reduction of disulfide bonds is performed using a proprietary semi-precious metal working electrode. The unique properties of the working electrode allow for complete reduction of all disulfide bonds of the tested proteins and peptides. Furthermore, a special electrochemical method based on square-wave potential pulses was developed and applied for efficient and stable reduction.
Insulin, a small protein of 5733 Da containing 3 disulfide bridges was used as model protein for system evaluation. The results are compared with the previous approach exploiting a conductive diamond working electrode. Reduction of other peptides and proteins such as Somatostatin with one disulfide bond (1638 Da) and α-Lactalbumin with four bonds (14 178 Da) will be shown to demonstrate the power of electrochemical S-S bond reduction.

The data were acquired using high resolution Fourier transform ion cyclotron resonance mass spectrometer (FT ICR) that allowed for accurate molecular weight determination of intact protein before and after reduction. Additionally, high mass resolving power was necessary for unambiguous identification of a peak originated from Somatostatin and its reduced form.

The electrochemical cell can be positioned before or after the HPLC separation, i.e., in an EC/LC/MS or LC/EC/MS set-up, resulting in a fully automated platform for fast characterization of protein/peptide based biopharmaceuticals.

PMo-101

11:10 – 12:20

Isotope dilution LC-ESI/MS/MS method for the determination of phenylthiohydantoin (PTH)-amino acids derived from Edman degradation products of proteins

Ryo Satoh, Takaaki Goto, Seon Hwa Lee, Tomoyuki Oe

Tohoku University

Keywords:
protein, absolute quantitation, Edman degradation

Novel aspects:
The first isotope dilution LC-ESI/MS/MS method for the determination of phenylthiohydantoin-amino acids derived from Edman degradation products of proteins has been developed.

Abstract:
[Objective]
Edman degradation reaction, reported in 1950, is a well-known method for amino acid (AA) sequencing in a peptide. It was used almost exclusively before mass spectrometry (MS) -based sequencing appeared. The methodology is based on the stoichiometric labeling of *N*-terminal α-amino group by Edman reagent (phenylisothiocyanate, PITC) followed by the quantitative degradation to release the *N*-terminal AA as the phenylthiohydantoin derivative (PTH-AA) . Since the methodology is robust, we have been studying the possibility to quantitate not only peptides, but also proteins as the PTH-AAs. That could enable to perform absolute quantitation of proteins without use of protein standards. To prove this idea, we have already confirmed the concept using matrix assisted laser desorption ionization (MALDI) -time of flight (TOF) /MS [1] . Here, we demonstrate the first isotope dilution liquid chromatography (LC) -electrospray ionization (ESI) /tandem mass spectrometry (MS/MS) method for the determination of PTH-AAs derived from Edman degradation products of proteins. The long-term stability experiments of twenty PTH-AAs and the applications for model proteins/peptides have been also performed.
[Experiments]
High-performance liquid chromatography (HPLC) was carried out using an UltiMate 3000 HPLC series (Dionex Co.) with an Inertsil ODS-4 column (150 × 2.1 mm i.d., 3 μm, 100 &#8491 ; , GL Sciences, Inc.) . A TSQ Vantage triple stage quadrupole mass spectrometer (Thermo Fisher Scientific Inc.) with an ESI source was used for selected reaction monitoring (SRM) . The long-term stability of PTH-AAs was examined in four different storage conditions (25% (v/v) aqueous trifluoroacetic acid, pH3, pH7, and pH11 buffers) at 4 &#8451 ; followed by monitoring with HPLC/UV. A mixture of twenty AAs was allowed to react with PITC and [$^{13}C_6$] -PITC to obtain standards and the corresponding internal standards, respectively. Human serum albumin (HSA) , angiotensin II, α -casein, lysozyme, ribonuclease A, pepsin, and insulin were used as model proteins/peptides.
[Results & Discussion]
The LC conditions were optimized in terms of effective ESI and chromatographic separation of the isovalent mass amino acids, such as Leu vs. Ile. Using acidic mobile phase (0.1% formic acid/water-acetonitrile) , all PTH-amino acids were well ionized and the base line separation between PTH-Ile and PTH-Leu was also achieved. Since the optimal fragment ion for SRM was selected to each PTH-AA/ [$^{13}C_6$] -PTH-AA, simultaneous analysis of twenty PTH-AAs was attained as stable isotope dilution method. The calibration curve of each PTH-AA showed good linearity (r^2 = 0.995-1.000) throughout the range of 1-100 pmol/injection. Also, the storage condition of each PTH-AA (at least 90 % within a week) was optimized for long-term storage as solutions. From the experiments using the seven model proteins/peptides, corresponding PTH-AAs released from the *N*-terminal amino acids were observed selectively. The yields of PTH-AAs derived from proteins were almost quantitative even from the largest model protein (77%, HSA, 66 kDa) . Since this stable isotope dilution LC-ESI/MS/MS method is robust and selective enough for the determination of PTH-AAs, it could be used for absolute quantitation of proteins even without the use of protein standards.
[1] Oe T., Maekawa M., Satoh R., Lee S.H., and Goto T., *Rapid Commun. Mass Spectrom.***24**, 173-179 (2010) .

PMo-102
13:30 – 14:40

Mechanistic Study on Non-enzymatic Isomerization of N-terminal Amino Acids by Endogenous Aldehydes

Ryo Kajita, Takaaki Goto, Seon Hwa Lee, Tomoyuki Oe

Tohoku University, Sendai, Japan

Keywords:
isomerization, N-terminal amino acid, ketimine

Novel aspects:
The mechanism of non-enzymatic isomerization of N-terminal amino acid mediated by endogenous aldehydes has been revealed to involve a ketimine intermediate.

Abstract:
[Objective]
The aldehydes can easily react with other biomolecules containing a nucleophilic functional group. There have been a lot of studies on the aldehyde-mediated biomolecular modifications because a large number of different aldehydes have been detected *in vivo* and most of them have intrinsic bioactivities. In the reaction of the bioactive peptide, angiotensin II (Ang II : H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) with the lipid peroxidation derived aldehydes, we have recently identified a pyruvamide-Ang II formed via oxidative decarboxylation of N-terminal Asp as the most abundant product along with other modified Ang IIs. It was suggested that the pyruvamide-Ang II was produced by deamination of N-terminal Asp after the formation of Schiff base intermediate and subsequent decarboxylation. In addition, the non-enzymatic stereo-isomerization of N-terminal amino acid was observed in the reaction between model peptides and various endogenous aldehydes under a physiological condition. We reasoned that the ketimine formed by tautomerization of the Schiff base intermediate can add a hydrogen atom non-stereo-specifically, leading to the isomerization of N-terminal amino acid. To clarify this mechanism, the model peptide was reacted with aldehyde in the absence or presence of heavy water and the reaction mixture was analyzed by LC/ESI-MS and MS/MS. The possibility of N-terminal amino acid isomerization of the protein was also examined.

[Experiments]
The model peptide (L-FMRF-amide : H-Phe-Met-Arg-Phe-NH_2) was incubated with pyridoxal 5'-phosphate (PLP) in phosphate buffer (pH7.4) at 37°C. LCQ-DECA (Thermo Fischer Scientific Inc.) was employed for the analysis of the reaction mixture. The chromatographic separation of reaction products was carried out on an Inertsil ODS-3 (150 × 2.1 mm i.d., 3 μm ; GL sciences Co. Ltd) with water and acetonitrile containing 0.1% formic acid as mobile phases at 0.2 mL/min. The same reaction was also done in the heavy water. As for the protein model, human serum albumin (HSA) and its N-terminal tetra-peptide (L-DAHK : H-Asp-Ala-His-Lys-OH) were used. HSA was incubated with PLP, followed by a trypsin digestion. The tryptic peptide was analyzed by LC/ESI-MS after derivatization with dansyl chloride.

[Results and Discussion]
LC/ESI-MS analysis of the reaction between L-FMRF-amide and PLP revealed the presence of five products together with D-FMRF-amide which was formed by isomerization of N-terminal Phe. Four products had an identical protonated molecule (MH^+) which corresponded to an increase in mass of 229 Da (+ [PLP-H_2O] , Schiff base) . The MH^+ of another product was consistent with the deamination of L-FMRF-amide. *MS/MS* and $(MS)^3$ analyses further revealed that both modifications occurred at N-terminal Phe, and that the intermediate of N-terminal isomerization could be Schiff base. Results of the reaction in the heavy water showed that deuterium was added to D-FMRF-amide and two Schiff bases, which was confirmed by their isotopic compositions (ratios of heavy to light isotopes) . This suggests the formation of ketimine intermediate during the reaction. Thus, the isomerization of N-terminal amino acid occurred through a mechanism involving non-stereo-specific addition of a proton to ketimine. The detection of deaminated product generated by an irreversible hydrolysis of ketimine intermediate also supports this isomerization mechanism. The isomerization of N-terminal Asp was subsequently observed in the reaction of L-DAHK, equivalent to N-terminal sequence of HSA, with PLP. Furthermore, D-DAHK fragment was detected when HSA was reacted with PLP, followed by tryptic digestion. These results suggest that the isomerization of N-terminal amino acid can occur in the protein through the same mechanism.

PMo-103
11:10 – 12:20

Peptide fragmentation upon low and high energy CID in ESI and MALDI mass spectrometry: A statistical approach

Christine Enjalbal, Mathieu Dupre, Sonia Cantel, Jean Martinez

University of Montpellier 2

Keywords:
Peptide, fragmentation, low and high energy dissociation, MS/MS, CID, gas-phase rearrangement, De Novo sequencing

Novel aspects:
Statistical approach to question peptide fragmentation upon CID activation in ESI and MALDI mass spectrometry from an home-made non biaised peptide set covering a wide structural diversity .

Abstract:
Proteomics has emerged as a major tool in understanding protein functions as well as protein-protein interactions at the cellular level. Mass spectrometry, through "top-down " or "bottom-up " strategies, constitutes one of the best method to identify protein primary sequences. In the course of these analytical protocols, MS/MS experiments are conducted to induce fragmentations of the polyamide skeleton. Such data, recorded upon high or low energy dissociation conditions, ideally enable to decipher the amino acid sequence. However, the extent of the gathered information varies strongly according to structural and instrumental conditions (nature of the peptide chains, type of mass spectrometer, activation method, fragmentation tuning,...) . Following a statistical approach, we investigated the low and high energy collision induced dissociation (CID) behaviors in ESI and MALDI mass spectrometry (ESI-Q-q-Tof and MALDI-Tof-Tof instruments) of various synthetic peptides (300 sequences of variable compositions and lengths mimicking proteolytic peptides obtained by the action of various enzymes such as trypsin, Lys-N, Glu-C, ...) .[1,2] Our attention was particularly focussed on singly charged peptides that often failed to be identified by sequencing algorithms. Only half of the studied compounds showed charge directed dissociations in accordance with the mobile proton model producing fragment ions directly related to the primary sequence. For the peptides that did not exhibit the expected fragment ion series, alternative dissociation behaviors issued from complex rearrangements were evidenced thus providing a more comprehensive fragmentation model suitable for more efficient automated MS/MS spectra interpretation. Such remarkable gas-phase behaviors were mainly attributed to the presence of basic residues (Arg, His, Lys) and to their relative localisation within the peptide sequences.[3,4]

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2. N. Shenar, N. Sommerer, J. Martinez, C. Enjalbal, *J. Mass Spectrom*. **44**, 621-632 (2009)
3. M. Dupre, S. Cantel, P. Verdié, J. Martinez, C. Enjalbal, *J. Am. Chem. Soc. Mass Spectrom*, 2011, **22**, 265-279.
4. M. Dupre, S. Cantel, J. Martinez, C. Enjalbal, *J. Am. Chem. Soc. Mass Spectrom*, 2012, **23**, 330-346.

PMo-104 Rapid Asp-selective Acid Hydrolysis for Proteomic Workflows

13:30 – 14:40

Joe R Cannon, Catherine Fenselau

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Keywords:
Middle-down, Middle-out, Orbitrap, Acid Digestion

Novel aspects:
Presented is a novel method for enhanced analysis of middle-out and bottom-up sized peptides using high resolution accurate mass on a hybrid mass spectrometer.

Abstract:
Microwave-supported acid hydrolysis exploits kinetically selective cleavage at Asp residues to provide very rapid protein cleavage, 10sec to 20 min, depending on sample complexity. We report the evaluation of this technique in three different proteomic workflows. First, its utility is demonstrated for digestion in solution, using a system poorly suited for tryptic digestion, the human ribosome. The high abundance of Lys and Arg residues required to interact with the phosphate backbone of ribosomal RNA makes the average peptide length following tryptic digestion less than 5 residues; too small for effective data dependent analysis (Cannon et al, JPR 2010, 9 : 3886-90) . Second, the digestion method is shown to be effectively applied "in-gel " to bands of an electrophoretically fractionated whole cell lysate from human multiple myeloma cells. From eight excised bands, a total of 1124 peptides were identified representing 604 proteins. Finally, the same mixture was used to demonstrate increased efficiency in analyzing high and low mass peptide fractions partitioned about a 3000Da molecular weight threshold.
Longer peptide products are produced, on average, by this single residue cleavage reaction. This is illustrated *in silico* and in the laboratory by comparison of acid cleavage products from multiple myeloma with tryptic peptides from the same cancer cells. We and others have reported that many advantages are offered by longer peptides in middle-out proteomics strategies. A workflow has been designed and demonstrated to enrich peptides with masses exceeding 3000Da and increase their successful analysis by LC-MS/MS. Products of microwave-supported acid cleavage are fractionated into larger and smaller peptides using a 3 KDa molecular weight filter. Subsequent LC-MS/MS analysis of the larger peptides is further biased by setting data dependent scanning parameters to reject charge states less than z = 4. Successful implementation of this middle-out strategy also requires the use of optimized HPLC and an upgraded search program such as ProSight. In addition to the more informative higher mass peptides, the fraction enriched in lower mass peptides can be analyzed in classical high throughput bottom-up fashion.

PMo-105 Optimization of matrix preparation methods for MALDI-ISD of protein

11:10 – 12:20

Ryunosuke Iimuro, Mitsuo Takayama

Yokohama City University, Yokohama, Japan

Keywords:
MALDI-ISD, Matrix, Preparation, Protein

Novel aspects:
The optimization of matrix preparation methods for MALDI-ISD of protein was performed to obtain the abundant and steady ISD fragment peaks without sweet spots.

Abstract:
Introduction
The intensity and sharpness of MALDI-ISD fragment peaks of peptide and protein depend on the matrix used and co-crystallization of sample (matrix and analyte) solution. The procedures for analyte-matrix solution preparation are shown to greatly influence on the co-crystallization of sample solution. Depending on the co-crystallization, the ISD spectra were obtained with or without the "sweet spots". In order to get the reproducibility of ISD fragment ions, the ISD spectra have to be obtained by automatically and randomly scanning the sample crystal surface. Here we introduce 1-naphthol derivatives as novel matrix material to optimize the matrix solution preparations, by changing the fractional compositions of solvent and with or without added trifluoroacetic acid (TFA). Here we report the preparation methods of matrix solution for the MALDI-ISD of protein to attain co-crystallization without "sweet spots". The methods obtained would be applied to several kinds of protein and phosphorylated peptide.

Methods
The chemicals used as matrix were 5-amino-1-naphthol (5,1-ANL) and 5-hydroxy-1-naphthol (5,1-HNL), and were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). In order to determine the optimum matrix solvent compositions, different fractional compositions of solvents such as acetone, acetonitrile (ACN) and water (H_2O) were prepared at 10 mg/mL in acetone/H_2O (1 : 1), ACN/H_2O (1 : 1), ACN/H_2O (7 : 3), and ACN. In order to determine the influence of acid on the co-crystallization, matrix solution was prepared with and without added 0.1% TFA, i.e., the matrix solution at 10 mg matrix/mL solvent with added 0.1% TFA was prepared. Aqueous analyte solution of 10uL was premixed in a tube with the same volume of a matrix solution of 10uL. A 1 μL of sample solution was deposited onto a MALDI sample plate and the solvents were removed by drying in air at room-temperature. Analyte used for the optimization was horse cytochrome c (Mr12360).

Results and discussion
Of the different matrix solvent compositions tested, the composition of acetone /H_2O (1 : 1) for whichever matrix was not suitable for MALDI-ISD, because the abundance of ISD fragment peaks was very low. The composition of ACN/H_2O (7 : 3) was suitable for MALDI-ISD, while ACN/H_2O (1 : 1) was not suitable for MALDI-ISD. The solubility of matrix into solvent has been demonstrated to be important in MALDI sample preparation. Both matrices 5,1-ANL and 5,1-HNL have better solubility in the ACN/H_2O (7 : 3) than ACN/H_2O (1 : 1). As a result, ACN/H_2O (7 : 3) was most suitable for automatically and randomly scanning the sample surface. It was found that addition of TFA into the matrix solution showed a remarkable influence on the quality of the ISD spectrum. The matrix solution of 5,1-ANL prepared with TFA was not suitable for MALDI-ISD of protein, because the abundance of ISD fragment peaks was absolutely lower than when the solution without added TFA was used, while $[M+H]^+$ peak was absolutely high in the matrix solution with added TFA. On the other hand, the solution of 5,1-HNL prepared with TFA was suitable for MALDI-ISD. The abundance of both $[M+H]^+$ and ISD fragment peaks was absolutely high compared with the solution without TFA. In conclusion, we have presented optimized methods of matrix preparation procedures for MALDI-ISD of protein, i.e., ACN/H_2O (7 : 3) without added TFA for 5,1-ANL and ACN/H_2O (7 : 3) with added 0.1 % TFA for 5,1-HNL. The optimized matrix preparation methods obtained here would be applied to several different proteins and a tetra-phosphorylated peptide. The MALDI-ISD spectra obtained with the optimized matrix preparation methods showed the informative ISD fragment ions for the analysis of sequencing and secondary structural characteristics.

PMo-106
13:30 – 14:40

Comparative Proteomic Analysis of Virulence Variations in Xanthomonas Campestris pv. Campestris Strain 17, 11 and P20H

Tao-Shan Chang, Chien-Chen Lai

National Chung Hsing University, Taichung, Taiwan/Institute of Molecular Biology College of Life Science

Keywords:
Xanthomonas campestris pv. campestris / black rot / 2 D electrophoresis / LC-MS/MS

Novel aspects:
To our knowledge, this is the first report of using the proteomic strategy to analysis of virulence variations in xanthomonas campestris pv. campestris strain 17, 11 and P20H.

Abstract:
Xanthomonas campestris pv. campestris (*Xcc*) is a Gramnegative plant pathogenic bacterium causing black rot in crucifers, resulting in tremendous loss in agriculture. The ability of *Xcc* to infect plants successfully depends on certain factors including extracellular enzymes (such as protease, endoglucanase, pectinase and mannanase) , exopolysaccharides (xanthan gum) and biofilm production. A newly isolated pathogenic Xc17 from an infected cabbage leaf for some phenotypic characteristics have previously been implicated as factors contributing to pathogenicity. A wild-type strain of *X. campestris*,Xc11, was found to have lost its pathogenicity spontaneously after frequent subculturing for years. This non-pathogenic derivative, designated Xc11A. *X. campestris* P20H was a non-mucoid mutant previously isolated from Xc11A by mutagenesis with nitrous acid. Recently the proteomics have been developed more widespread, the techniques can offer insights into the quantity and quality of the final gene products. In this study, we aim to elucidate the physiological and molecular mechanism response to virulence variations in *xanthomonas campestris* pv. campestris strain by using proteomics method. Total proteins of intracellular and extracellular were extracted for comparative proteomics analysis via 2 D electrophoresis and LC-MS/MS analysis. The result shows several differentially expressed proteins spots of bacterial proteome in Xc17, 11 and P20H. These identified proteins may be helpful in elucidating the molecular basis of virulence variations in *Xcc*.

Poster Session

Monday, 17th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 6: Novel Approaches in Proteomics Analysis

PMo-107 Proteomic Evidences for Starch Biosynthetic Pathway in Rice Endosperm

11:10 – 12:20

Chih-Wei Liu, Chien-Chen Lai

Institute of Molecular Biology,National Chung Hsing University, Taiwan

Keywords:
Rice / Starch biosynthesis / Proteomics / Gluconeogenesis

Novel aspects:
Novel starch biosynthesis pathway in developing rice endosperm has proven and demonstrated in our study.

Abstract:
Rice (Oryza sativa) is one of the most important cereals in the world. The starch reserves of the rice grains generally contain more than 75% amylopectin ; however, the abundance of amylose can affect the properties of starch in the grains. In this study, we use the Tainung 67 (TNG67) and its sodium azide-induced mutant SA0419 as the investigated materials. The low amylose content in SA0419 grains (8 %) is apparently different from TNG67 grains (20%) . Moreover, the superior efficiency of starch biosynthesis and shorter developing period are observed in SA419. We want to resolve the regulated mechanisms of starch biosynthesis in rice endosperm from protein expression level. Endosperm proteome of the grains of 14 days after anthesis (DAA) were extracted and separated by 2-DE. MS-based protein identification strategy was used to analyze the total proteome and differential-expression proteome of TNG67 and SA0419. The higher abundance of enzymes in SA0419 was used to produce the more α-D-glucose-1 P, and high level of starch biosynthesis enzyme were generated to produce the ADP-glucose and to accelerate the accumulations of starch. Furthermore, lack of granule-bound starch synthasein SA0419 is the major key factor to cause the low amylose content. Overall, the starch biosynthesis model in the rice endosperm was presented in this study. The increased in gluconeogenesis pathway and reduction of the other metabolism pathway in SA0419 elevated the substrate contents for starch biosynthesis.

PMo-108
13:30 – 14:40

MALDI-TOF-MS-based proteomics for urinary biomarker discovery in gamma-irradiated mouse

Keita KIRIYAMA, Susumu YOSHIOKA, Daisuke IIZUKA, Hidehiko KAWAI, Shunsuke IZUMI

Hiroshima University, Hiroshima, Japan

Keywords:
Biomarkers, Radiation, Hepcidin, Adipsin, Deglycosylation

Novel aspects:
1) Hepcidin 2 as potential biomarker for radiation exposure. 2) Adipsin was deglycosylated with radiation injury.

Abstract:
On March 11, 2011, a series of disasters at Fukushima in Japan killed thousands of people, left hundreds of thousands of people homeless and nuclear accidents exposed large numbers of people to ionizing radiation. It is now necessary to develop biomarkers for radiation dose assessment that can be used for mass screening in the event of a radiological mass casualty incident. High-throughput appliances that could evaluate such incident are expected as being employed to guide triage and subsequent therapeutic choices. Our aim of this study is to develop a high-throughput, sensitive biodosimetry technique which can be applied for the early triage of a radiation emergency medical system.

We employed a proteomic approach to analyzing urinary proteins, using mice as an animal model. The urine samples were collected from the mice which were exposed to total-body radiation (4.0-6.0Gy) produced by the ^{137}Cs gamma-ray source. The urine was separated by HPLC and/or SDS-PAGE and analyzed by MALDI-TOF mass spectrometry using Ultraflex TOF/TOF (Bruker Daltonics) and AXIMA-QIT (Shimadzu).

We identified hepcidin 2 as potential biomarker for radiation exposure. The urinary concentration of hepcidin 2 was increased at 24 hours after gamma-irradiation in a dose-dependent manner, which coincides with the previous observation. The blood hepcidin 2 levels was also increased at 8 hours after gamma-irradiation, this is the first observation of the increase of urinary hepcidin 2. In addition, a 27-kDa protein was increased at 24 hours after gamma-irradiation in a dose-dependent manner. The 27-kDa protein (SDS-PAGE) was identified by peptide-mass fingerprinting and MS/MS analysis as adipsin. Adipsin is a glycoprotein of 44- and/or 37-kDa,[1)] and a serine protease homolog, which is synthesized and secreted by adipose cells and is found in the bloodstream. These results indicate that the sugar chain of urine adipsin was cleaved by radiation exposure.

Thus, urinary metabolites may be a potential biomarker for acute ionizing radiation exposure. Further studies may be warranted for the development of new biodosimetry methods for radiation exposure.

1) B. S. Rosen *et al., Science*, **244**, 1483-1487 (1989).

PMo-109
11:10 – 12:20

LDI-MS on nanostructured materials for sensitive and efficient protein digest characterization

Mathieu Dupre[1], Sonia Cantel[1], Yannick Coffinier[2], Rabah Boukherroub[2], Jean Martinez[1], Christine Enjalbal[1]

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Keywords:
LDI-MS, Nanostructured silicon, Tryptic peptides, PMF, Peptide sequencing

Novel aspects:
Nanostructured silicon platforms for direct high-throughput robust protein digest characterization.

Abstract:
Laser desorption/ionization mass spectrometry (LDI-MS) using specific inert surfaces to promote ion formation has been widely investigated the last decade [1]. In addition to porous silicon through the original DIOS technique, different materials were tested as potent LDI-promoting agents, such as metals, carbon-based structures, porous particles, nanomaterials and more recently, ordered three-dimensional silicon architectures. All of these matrix-free methods are now referred as Surface Assisted Laser Desorption Ionization-Mass Spectrometry (SALDI-MS,http : //www.iupac.org).
Up to now, disposable ready-to-use target plates (MassPREP™, QuickMass™ and NALDI™) can be purchased for SALDI analysis of small molecules. In order to overcome the limitations encountered with the use of such commercially available probes (surface pollution affecting detection sensitivity and reproducibility), we explored a variety of inert silicon-based UV-absorbing materials that were presenting different physico-chemical properties for the analysis of peptides [2-4]. Both material architecture (amorphous powders, structured particles, structured surfaces) and material hydrophilic/hydrophobic character tuned by specific chemical derivatization (oxidation, silanization) were probed as crucial parameters for achieving efficient and robust detection of an home-made array of model peptides covering a wide structural and mass diversity (50sequences, m/z 500-2000 Da). Through this set of experiments, we were able to compare the performances of all investigated silicon-based supports, especially taking into account peptide detection sensitivity (down to femtomolar concentrations) and reproducibility/repeatability (intra-spot/inter-spot signal variations) as well as the method robustness (easy sample preparation, need for ionization additives, straightforward sample deposit depending on material wetting properties, ionization source tuning using conventional MALDI-TOF/TOF instrument). Having illustrated the capability to achieve both peptide detection and sequencing on these ionizing surfaces in the same run, high-throughput identification of protein tryptic digests by a rapid MS profiling and subsequent MS/MS analyses was undertaken. Tryptic digests from Cytochrome C, Beta-casein, BSA and Fibrinogen were analyzed in the femtomolar range (for instance, from 50 fmol for Cytochrome C down to 2 fmol for Fibrinogen for a nanostructured silicon surface). Comparison of the MS and MS/MS data with those obtained with sample conditioned in organic matrix [5,6] and on commercial target (NALDI™) demonstrated a great behavior for low mass responses.
We thus demonstrated the capability of LDI on nanostructured silicon supports to be a complementary method to MALDI Peptide Mass Fingerprinting (PMF) ensuring determination of peptide molecular weights and sequences for more efficient protein database searches in proteomic workflow.

[1] Peterson DS. *Mass Spectrom. Rev*. 2007 ; **26** : 19-34.
[2] Shenar N, Martinez J, Enjalbal C. *J. Am. Soc. Mass Spectrom*. 2008 ; **19** : 632-44.
[3] Shenar N, Cantel S, Martinez J, Enjalbal C. *Rapid Commun. Mass Spectrom*. 2009 ; **23** : 2371-9.
[4] Dupre M, Coffinier Y, Boukherroub R, Cantel S, Martinez J, Enjalbal C. *J. Prot*. 2012 ; **75:**1973-1990.
[5] Dupre M, Cantel S, Verdie P, Martinez J, Enjalbal C. *J.Am. Soc. Mass Spectrom*. 2011 ; **22** : 265-79.
[6] Dupre M, Cantel S, Martinez J, Enjalbal C. *J. Am.Soc. Mass Spectrom.*, 2012 ; **23** : 330-46.

PMo-110

13:30 – 14:40

Evaluation of peptide adsorption-controlled liquid chromatography-mass spectrometric (PAC-LC-MS) method to quantify peptides in biological fluids

Ryoya Goda[1], Ryo Yokoyama[2], Nobuhiro Kobayashi[1]

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Keywords:

peptide adsorption-controlled liquid chromatography, tandem mass spectrometry, biological fluids, simultaneous quantitation

Novel aspects:

PAC-MS/MS method has the potential for highly sensitive and quantitative analysis of peptides in biological fluids.

Abstract:

Introduction

Endogenous peptides in biological fluids have actively been investigated as biomarkers for discovery of clues to underlying cause of disease, for early and correct diagnostics, and for effective therapy against various diseases. However, both discovery of functional peptides and analysis of their functions are difficult because many peptides exhibit their functions only at concentrations in the low picomolar range. Recently, there is a compelling need for a methodology based on high-performance liquid chromatography-mass spectrometry (LC-MS) for the quantitation of peptides in biological fluids, because LC-MS methods possess considerably higher selectivity based on the mass-to-charge (*m/z*) ratios in addition to chromatographic separation, and better reproducibility in comparison with immunological methods.

We found that the adsorption capacity of a peptide to the column packing changed abruptly and reversibly across a specific amount of organic solvent (the critical point, $f_n = 1$) in the peptide solution. Circular dichroism spectral analysis suggested that the abrupt change in the adsorption capacity was caused by the conformational change induced by the organic solvent in the peptide solution. This solvent-induced conformational change was found to be reversible across the critical threshold. Based on this property, we developed a new gradient system, which is named peptide adsorption-controlled liquid chromatography (PAC-LC), for quantitative analysis of peptides in the solution with a higher fn value than 1 ($f_n > 1$). PAC-LC enables quantitation of peptide in such solutions without any loss of sensitivity due to peptide adsorption to various materials, and found to be superior to a standard LC system in terms of precision, loading volume onto the system, sensitivity, and the simultaneous and quantitative analysis of peptides with good precision. Then, we have evaluated the usefulness of PAC-LC coupled with tandem mass spectrometer (4000 QTRAP and TripleTOF5600) for quantitation of peptides in biological fluids such as plasma and cerebrospinal fluid (CSF).

Method

Using PAC-LC coupled with 4000QTRAP, quantitation of peptides in biological fluids was carried out using multiple reaction monitoring (MRM) mode. In the case of TripleTOF 5600, quantitation of peptides was carried out using either TOF-MS scan or TOF-MS/MS scan mode. During the evaluation, stable isotope-labeled peptides were also evaluated as the internal standards to minimize the influence of matrix on the reproducible quantitation. The calibration curves for endogenous peptides were drawn from the standard calibration solutions.

Preliminary Data

The results of the study suggest that PAC-LCMS method is a powerful tool to quantify peptides in biological fluids. For example of analysis using MRM mode, PAC-LC-MS method enables simultaneous quantitation of amyloid β peptides (Aβ) 1-38, 1-40, 1-42 and 1-43 in dog CSF with good reproducibility. The basal concentration of Aβ 1-38, Aβ 1-40, Aβ 1-42 and Aβ 1-43 in dog CSF is found to be approximately 300, 900, 200 and 30 pM, respectively. Also, stable isotope labeled Aβ used as the internal standard can minimize the influence of CSF matrix on the reproducible Aβ quantitation. In the case of TripleTOF 5600, calibration curves from the standard calibration solutions were successfully constructed in the concentration range from 10 pM to 1000 pM for various peptides using TOF-MS scan mode. For TOF-MS/MS mode, the lower limit of quantitation was expected to be lower than 10 pM. The obtained results suggest that PAC-LCMS coupled with TripleTOF5600 have easily applicability to the quantitation of peptides in biological fluids.

PMo-111 Comparative proteomics of CD133-positive liver cancer stem cells

11:10 – 12:20

Sheng-Ta Tsai[1,2], Chih-Chiang Tsou[3], Wan-Yu Mao[1], Wei-Chao Chang[1], Wen-Lian Hsu[3], Chia-Ning Shen[1,2], Chung-Hsuan Chen[1,2,4,5]

[1]Genomics Research Center, Academia Sinica, Nankang, Taipei, Taiwan, [2]Institute of Biochemistry & Molecular Biology, National Yang-Ming University, Taipei, Taiwan, [3]Institute of Information Science, Academia Sinica, Nankang, Taipei, Taiwan, [4]Department of Chemistry, National Taiwan University, [5]Institute of Atomic & Molecular Sciences, Academia Sinica

Keywords:
Hepatocellular carcinoma / LC-MS/MS / Liver cancer stem cells / Label-free quantitation

Novel aspects:
The proteomes of CD133+ liver cancer stem cells, CD133- liver cancer cells and normal human hepatocytes were investigated using two label-free methods.

Abstract:
CD133-positive liver cancer stem cells, which are characterized by their resistance to conventional chemotherapy and their tumor initiation ability at limited dilutions, have been recognized as a critical target in liver cancer therapeutics. In the current work, we investigated the proteome of CD133-positive liver cancer stem cells for the purpose of identifying unique biomarkers that can be utilized for targeting liver cancer stem cells. The subpopulation of Huh 7 hepatoma cells that express the glycosylated CD133 antigen was sorted and compared with normal hepatocytes and CD133-negative hepatoma cells. Label-free quantitation was performed by ID-based Elution time Alignment by Linear regression Quantitation (IDEAL-Q) . The results showed that 151 proteins were differentially expressed in the CD133-positive hepatoma cells when compared with CD133-negative cells. 21 up-regulated plasma membrane proteins were identified in the CD133-positive hepatoma cells, suggesting potential surface biomarkers for liver cancer stem cells. We validated the expression of some of the up-regulated membrane proteins either by MaxQuant software or by RT-PCR, immunoblot, and immunofluorescent analysis. We identified annexin A 1 and annexin A 3 were highly expressed in the CD133-positive hepatoma cells. These findings confirmed that mass spectrometry-based label-free quantitative proteomics can be used to gain insights into liver cancer stem cells.

PMo-112
13:30 – 14:40

Quantitative proteomics approach with isotope dimethyl labeling for protein pathway analysis using high resolution mass spectrometry

Ping Sui, Konstantin Artemenko, Jonas Bergquist

Uppsala University, Uppsala, Sweden

Keywords:
Quantitative proteomics, Isotope dimethyl labeling, Mass spectrometry, Rat spinal cord

Novel aspects:
Our method provide a fast, simple and inexpensive way for protein pathway analysis in quantitative proteomics, especially for small animal sample, for example rat spinal cord.

Abstract:
Protein expression in the cell could be easily affected by, among other things, drugs, disease or stress. The change in protein expression may activate a response bio-mechanism and influence expression of other related proteins, which makes corresponding pathways up or down regulated. By quantifying the protein expression in biological tissues we can identify the activated pathways, which are caused by or related to a certain disease or clinical symptoms. In this study, we compared the protein expression level in contralateral dorsal and contralateral ventral parts of the spinal cord in naive rats. We use dimethyl isotope labels to globally label the tryptic peptides for relative quantification. Proteins were extracted from homogenized rat spinal cord tissues, then digested, labeled and analyzed by LC-MS/MS using FT-ICR instrument. Sample preparation protocol and the acquisition parameters of LC-MS/MS were optimized, especially for relative quantitative proteomics analysis in spinal cord sample. Stability and repeatability of the method were evaluated as well. Related bio-pathway analysis was performed using IPA from Ingenuity.

The results show that 17.2% of the mapped proteins are related with the nervous system development and functions and most of them have higher expression level in the contralateral dorsal region than in the contralateral ventral region. Relevant pathways in the nervous system can be identified with a high confidence value, for example GABA receptor signaling and protein ubiquitination pathways. The efficiency of the isotope dimethyl labeling is stable and data acquisition of FT-ICR is robust for relative quantitative proteomics analysis. This method could be applied to the study of neuro-related dysfunctions e.g. neuropathic pain in the central nervous system. Due to the complexity of biological samples and the high dynamic nature of proteome, large number of samples and replicates are needed to enhance the confidence of the inferences from the experiment.

PMo-113
11:10 – 12:20

MS-based proteogenomics and comparative proteomics help to identify novel key proteins in organisms with newly sequenced genomes

Rainer Cramer[1], Laurence V Bindschedler[1], Dana M Gheorge[2], Liam J McGuffin[1], Celia J Smith[1], Clara M Pliego Prieto[2], Pietro D Spanu[2]

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Keywords:
proteogenomics, pathogen effectors, protein interaction, host-pathogen interaction, comparative proteomics

Novel aspects:
We will demonstrate the power of MS-based proteogenomics and comparative proteomics in annotating newly sequenced genomes and identifying and characterizing novel proteins not seen before, analysing Blumeria graminis.

Abstract:
The barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) is an economically important fungal obligate pathogen in temperate regions around the world. Large-scale MS-based proteomics has been successfully employed for a proteogenomic approach in validating the annotation of its recently assembled genome. A comparative MS-based analysis of the proteomes of Blumeria spore and hyphae cells to Blumeria haustoria cells (i.e. the cells involved in infection and direct interaction with the host through nutrient uptake and elicitor delivery) allowed the identification of Blumeria effector candidate (BEC) proteins unique to haustoria cells. These proteins are secreted, most have little or no sequence similarity to known enzymes and are likely to represent novel structural folds as they have poor predicted 3D models. Some of these proteins were shown to have a biological function in the infection process. Their Blumeria and barley interactors are being sought by "pull down " affinity chromatography using expressed tagged recombinant BEC proteins as baits.
We will demonstrate the power of MS-based proteogenomics and comparative proteomics in annotating newly sequenced genomes and identifying and characterizing novel proteins not seen before.

PMo-114 MALDI msn analysis of protein oxidation in major diseases

13:30 – 14:40

Lina Sellami[1], Claude Villard[1], Kamel Mabrouk[1], Therese Schembri[1], Didier Gigmes[1], Matthew Openshaw[2], Omar Belgacem[2], Daniel Lafitte[1]

[1]Aix Marseille Universite, Marseille, France, [2]Shimadzu, Manchester, United Kingdom

Keywords:
MALDI, MSn, single isotope selection, protein modification, oxidation

Novel aspects:
MALDI MSn of complex protein patterns with single isotopic selection at various MS/MS stages

Abstract:
Introduction

Production of reactive oxygen species (ROS) is a characteristic feature of a variety of (patho) physiological processes. It is recognized that ROS are makers of disease since they can induce deleterious effects, e.g. oxidation of biomolecules such as lipids and proteins. Today, oxidative stress is thought to be involved in many pathologies ranging from neuronal (e.g. Alzheimer's and Parkinson's disease) and metabolic disorders (e.g. hypercholesterolemia and diabetes) to chronic inflammatory diseases (e.g. atherosclerosis and arthritis) and cancer.
In this context, protein carbonylation as the major form of protein oxidation is considered as universal indicator for oxidative stress of biological samples and products. During oxidative damage carbonyl groups can be generated directly from amino acids (e.g. Arg, Lys, Pro, Glu, Asp) or indirectly *via* Shiff's base (e.g. aldehydes) or Michael adducts (e.g. α,β-unsaturated carbonyls) of advanced lipid oxidation products (e.g. fragmented OxPLs) , which are oxidized to the carbonyl level.

The limited number of current data on the successful analysis of carbonylated proteins by mass spectrometry can be explained by the difficulty of characterizing oxidized proteins by conventional proteomic approaches. We present in this work top down and bottom up strategies using MALDI MS^n technologies that allow exhaustive characterization of protein and peptide modification.

Material and methods

For protein oxidation, protein and peptide stock solution were mixed with 5 mM NaOCl solution. The mixture was incubated for 15 min at 37 °C in a water bath. Following oxidation, the proteins and peptides were purified using a ZipTip microcolumn and eluted the matrix. 2 µl (approximately 3 µg) were applied either to the MALDI-TOF and MALDI-QIT-TOF target and allowed to dry at ambient temperature. MALDI spectra were acquired on a MALDI-TOF and on a high vacuum MALDI-QIT-TOF instrument (Shimadzu, Manchester, UK) . The control software (Launchpad, version 2.9.2) was used for fragment annotation and analysis. Protein identification was performed using mascot (http : //www.matrixscience.com) .

Results, discussion and perspectives

The MALDI-QIT-TOF technology was used to fragment oxidized peptides and proteins. Protein fragments were analyzed after digestion or directly in the source by in source decay. Ions were transferred to the ion-trap and submitted further to multiple cycles of MS^n using CID. The resolution for all the fragmentation stages (more than 15000 FWH) allowed selection of single isotopes selection and up to five cycles. Protein carbonylation generates modifications on lysine (-1 Da) , Threonine (-2 Da) , proline (+16 Da) or arginine (-43 Da) . Especially for lysine, the most abundant modification, selection of a single isotype in each MS/MS step has been a real benefit for unambiguous characterization of protein modification.

PMo-115
11:10 – 12:20

Quantitative Proteomics Using a Quadrupole-Orbitrap Mass Spectrometer (Q-Exactive): Application to the Analysis of Clinical Samples

Bruno Domon, Sebastien Gallien, Elodie Duriez

Luxembourg Clinical Proteomics Center CRP-Sante Luxembourg

Keywords:
quadrupole-orbitrap, quantification, proteomics, clinical application, targeted experiments

Novel aspects:
Novel quantification method to analyses peptides in complexe biological samples, leveraging the quadrupole-orbitrap instrument.

Abstract:
A hybrid quadrupole-orbitrap mass spectrometer offers unique capabilities, in term of resolution, mass accuracy, and acquisition speed that has enabled novel quantitative proteomics approaches. This instrument, combined with stable isotope dilution, allowed developing new methods to detect and quantify large sets of peptides in complex mixtures, such as urine and blood proteomic samples.

Quantification was performed either on the precursor ions, using the selection ion monitoring (SIM) mode, or on the fragment ions resulting from collision dissociation (i.e. reaction monitoring mode) .
The high resolution capability in both MS and MS/MS modes allows a dramatic improvement of the measurement selectivity, by effectively discriminating the signals of analytes from those of the background. In addition, the narrow m/z isolation window of the quadrupole, results in an increases of the dynamic range and the limit of detection, by gating only the ions of interest in the trapping device. This mode of operation enables reliable quantification over a wide dynamic range.

The sample preparation consisted in the precipitation of the proteins present in the urine samples, followed by trypsin proteolysis. The isotopically labeled synthetic polypeptide standards, comprising proteotypic peptides of the protein of interest and a universal reporter peptide were spiked into the sample prior to the proteolysis. Analyses were performed on a nano-HPLC system coupled with a hybrid quadrupole-orbitrap instrument (Q-Exactive, Thermo Scientific, Bremen) .

The MS/MS experiments were used to conduct in a targeted (SRM-like) mode, to quantitative reference peptides spiked in urine and plasma samples, and thus precisely measure the corresponding endogenous peptides. Various combinations of quadrupole isolation windows and orbitrap resolution settings were evaluated to determine their effect on the selectivity of the measurements. Other parameters such as the collision energy were also evaluated. The actual quantification was carried out post-acquisition by extracting the ion chromatograms of specific fragment ions. The co-elution of the traces was used to assess the "purity " of the ions measured, and a reference library of MS/MS spectra was used to systematically confirm the identity of the peptides analyzed.

The performance of SRM-like quantification was determined using urine samples, and benchmarked with the results obtained by SRM technique on triple quadrupole instrument. Experiments were performed on ten clinical samples spiked with a defined amount of isotopically labeled peptides representing four endogenous proteins. Time-scheduled experiments were performed on both instruments using the same precursor ion isolation window. The selectivity improvement due to higher resolution allowed a better discrimination of interferences in the MS/MS spectra. To scale-up the number of peptides targeted in one single LC-MS/MS analysis, acquisition parameters were adapted to keep a low duty cycle. It included relaxing the resolving power, and selecting several peptide precursors to generate a composite fragment ion spectrum (i.e. multiplexing capabilities) . The quality of the measurements was assessed in a second study comprising 200 isotopically labeled reference peptides spiked into urine tryptic digests. Experiments with different resolution (17k-70k) and quadrupole isolation windows (1 Th-100Th) were performed, including the acquisition in data independent mode. The selectivity for each set of experimental parameters provided a baseline for the establishment of acquisition methods generally applicable. The approach was applied to the precise quantification of potential biomarkers of recurrence of bladder cancer in urine and serum samples.

These results demonstrate the benefit of using the high resolution, accurate mass to increase the selectivity of quantitative assays in very complex matrices by a better discrimination of the interferences. The recent development of the acquisition software enabled the very precisely analysis of more than 500 peptides in one single experiment, thus dramatically easing the verification step of biomarkers studies.

PMo-116
13:30 – 14:40

Using Selected Reaction Monitoring to determine cell and drug specific profiles of caspase cleaved substrates in apoptosis

Kazutaka Shimbo[1,2], Gerald W Hsu[2], Huy Nguyen[2,3], Sami Mahrus[2], Jonathan C Trinidad[2], Alma L Burlingame[2], James A Wells[2,3]

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Keywords:
apoptosis, caspase, proteomics, quantification, selected reaction monitoring

Novel aspects:
Quantitative proteomics, SRM coupled with N-terminomics, approach could determine caspase-cleaved peptide profiles in different cancer cell lines and inducers of apoptosis. These may aid in the development of specific biomarkers.

Abstract:

Introduction

Apoptosis is a conserved pathway and has important roles in all metazoans. Dysfunction in apoptosis is critical to cancer cell survival and most cancer chemotherapies promote apoptosis. Apoptosis can be triggered by two pathways, extrinsic and/or intrinsic pathways. Caspases, cysteine-class proteases, are activated during apoptosis and cleave substrates after aspartic acid residues to deconstruct the cell. Different cancer drugs can induce apoptosis through different substrates and pathways, for instance, inhibiting protein kinases, topoisomerases or inhibiting the proteasome to induce the cellular stress. Moreover different cell lines show different response to these drugs for reasons that are often poorly understood. Here, we used a label-free quantitative proteomics approach called selected reaction monitoring (SRM) coupled with a positive enrichment N-terminomics approach to determine caspase-cleaved peptide profiles in different cancer cell lines and different inducers of apoptosis.

Methods

Three hematologic malignancy cell lines, T-cell leukemia (Jurkat cell line), B-cell lymphoma (DB cell line), and plasma cell multiple myeloma (MM1S cell line), were used for these experiments. Cells were grown to uniform density and treated with staurosporine (DB, Jurkat and MM1S) and either doxorubicin (DB and Jurkat) or bortezomib (MM1S). Cell viability and caspase activity were monitored during the cultivation to control for different rates of cell death. In the initial discovery phase, cells were harvested at approximately 25% and 50% cell viability, and enriched for N-terminal peptides (Mahrus et al, Cell. 2008, 134, pp866-876). Enriched samples were pooled, fractionated and analyzed by conventional LC/MS/MS (QSTAR Elite, AB/Sciex) and proteins and peptides were identified using ProteinProspector (University of California, San Francisco). The putative SRM transitions were generated from MS/MS spectrum of identified caspase-cleaved peptides. For the relative quantification by SRM, cells were treated with three different drugs (staurosporine, bortezomib and doxorubicin or MG132) and harvested at multiple time points between 100% viability and near complete death (0% viability). SRM (QTRAP 5500, AB/Sciex) was performed and data were processed using in-house perl scripts. Internal standards were included to control for experimental and analytical variability and permit relative quantitative comparisons of peptide intensity.

Results and Discussions

From the discovery runs using pooled samples, we identified a total of 2083 α-amine containing peptides from 1256 proteins. About one-third (674/2083) of cleavages followed aspartic acid, which were putative caspase cleavage sites, and 800 peptides (including different charge states, missed cleavages by trypsin, and methionine oxidations) were selected to generate SRM transitions. Interestingly, 90% of peptides could be detected at some level in all three cell lines using SRM analysis of individual apoptotic cell lysates from the discovery experiment. However, there are large differences in signal intensities of peptides depending on cell type and inducer. For example, in MM1S cells treated with the proteasome inhibitor bortezomib, levels of activating transcription factor-4 (ATF4) increase dramatically early in drug treatment and then decrease upon cleavage by activated caspases. Thus, caspase-derived cleavage products are a sensitive reflection of cell-type and drug-induced stress, and provide useful fingerprints for mechanism of drug action and response.

In summary, these studies reveal the remarkable sensitivity of caspase cleavage profiles for drug and cell-type. These data may aid in the development of specific apoptotic biomarkers for determining drug action, cell-type specific cell death, and possibly new targets and pathways for cancer drug treatments.

PMo-117
11:10 – 12:20

Rapid enzyme digestion system consisting of microwave and air bubble-mediated cooling.

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Keywords:
microwave, cooling, proteomics, digestion, glycan

Novel aspects:
enzyme reaction in the microwave system combined with air bubble-mediated cooling can drastically reduce reaction time while still maintaining the efficiency of the enzyme digestion.

Abstract:
Efficient enzymatic digestion of proteins is critical in the analysis of protein samples, in particular when global proteomic analysis is required. However, the traditional water-bath enzymatic digestion has always been the major rate-limiting step in the sample processing for proteomic analysis. In recent years, microwave has been applied to protein digestions, and demonstrated very rapid completion of most digestion reactions within several minutes. In this study, the new microwave-assisted enzyme digestion system was developed, employing a reaction chamber equipped with water-bubbling system by air supply. Air bubbles in the microwave chamber keep on slightly decreasing the temperature of the chamber, leading to the continual irradiation of the chamber by microwave pulses, which, in combination with air bubble-mediated cooling system, will maintain the repetitive radiation of microwave pulses and constant temperature in the chamber. This combination of microwave pulses and cooling system by air bubbles controlled the water chamber temperature with variation less than 0.5 &#8451 ; . We tested in-solution and in-gel digestion of BSA and transferrin with several enzymes such as trypsin and chymotrypsin. The enzymatic release of oligosaccharides (N-linked glycans) from glycolproteins was also attempted with PNGase F in the microwave system. All the enzyme reactions were carried out with 400W of microwave power output & cooling air-bubble system at 37 &#8451 ; for 10 min. The efficiency of trypsin or chymotrypsin digestion was determined by MALDI-TOF mass spectrometry (MS) with matrix pre-spotted plates. The homogenous and continual microwave radiation throughout the reaction time significantly reduced the number of miss-cleavaged peptides in protein digestion. The sequence coverage of more than 80 % by including one miss-cleavaged peptides of BSA has been achieved in most of repeated experiments with air-bubbling microwave water chamber. The constant irradiation by microwave pulses significantly reduced the miss-cleavaged peptides from BSA and transferrin when peptide mass fingerprinting by MASCOT search was used for the analysis. In contrast, microwave irradiation which was not constant and uniform mostly led to the incompletion of reaction. We analyzed multiple mass spectrometry spots on the sample plates, and the MS data were very similar among identical spots, indicating the consistency of data analysis. The digestion efficiency from 10-min reaction with microwave system in combination with air bubble-mediated cooling reached almost identical level of efficiency obtained from conventional overnight digestion in regular water-bath. N-linked glycans released by PNGase F were also analyzed by MALDI-TOF MS, demonstrating that just 10-min digestion reaction in the microwave chamber with bubble-mediated cooling system was enough to result in the digestion efficiency similar to that acquired from overnight reaction using conventional incubators. These results point out that enzyme reaction in the microwave system combined with air bubble-mediated cooling can drastically reduce reaction time while still maintaining the efficiency of the enzyme digestion.

PMo-118
13:30 – 14:40

Biomarker Discovery and Validation with Nonglycosylated Tryptic Peptides from N-linked Glycoproteins in Human Plasma

Ju Yeon Lee[1,2], Jin Young Kim[1], Gun Wook Park[1,4], Kyung-Hoon Kwon[1], Yeong Hee Ahn[1], Myeong Hee Moon[2], Young Ki Paik[3], Jong Shin Yoo[1,4]

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Keywords:
plasma, N-linked glycoproteins, label-free quantification, multiple reaction monitoring (MRM) quantification.

Novel aspects:
This work provides a targeted means of discovering and validating non-glycosylated tryptic peptides as biomarkers in human plasma, without the need for complex enrichment processes or expensive antibody preparations.

Abstract:
A simple mass spectrometric approach for the discovery and validation of biomarkers in human plasma was developed by targeting non-glycosylated tryptic peptides adjacent to glycosylation sites in an N-linked glycoprotein, one of the most important biomarkers for early detection, prognoses, and disease therapies. The discovery and validation of novel biomarkers requires complex sample pre-treatment steps, such as depletion of highly abundant proteins, enrichment of desired proteins, or the development of new antibodies. The current study exploited the steric hindrance of glycan units in N-linked glycoproteins, which significantly affects the efficiency of proteolytic digestion if an enzymatically active amino acid is adjacent to the N-linked glycosylation site. Proteolytic digestion then results in quantitatively different peptide products in accordance with the degree of glycosylation. The effect of glycan steric hindrance on tryptic digestion was first demonstrated using alpha-1-acid glycoprotein (AGP) as a model compound versus deglycosylated AGP. Second, nonglycosylated tryptic peptide biomarkers, which generally show much higher sensitivity in mass spectrometric analyses than their glycosylated counterparts, were quantified in human hepatocellular carcinoma (HCC) plasma using a label-free method with no need for Nlinked glycoprotein enrichment. Finally, the method was validated using amultiple reaction monitoring analysis, demonstrating that the newly discovered non-glycosylated tryptic peptide targets were present at different levels in normal and HCC plasmas. The area under the receiver operating characteristic (ROC) curve generated through analyses of non-glycosylated tryptic peptide from vitronectin precursor protein was 0.978, the highest observed in a group of patients with HCC. This work provides a targeted means of discovering and validating non-glycosylated tryptic peptides as biomarkers in human plasma, without the need for complex enrichment processes or expensive antibody preparations.

PMo-119
11:10 – 12:20

The application of biomarker discovery for atopic dermatitis and normalized spectral index based label free quantitation

Ki Na Yun[1], Gun Wook Park[1], Hye Kyoeng Min[1], Jong Shin Yoo[1], Seongmin Noh[2], Chang Ook Park[2], Kwang Hoon Lee[2], Jing Young Kim[1]

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Keywords:
Proteomics, label free, Biomarker, atopy

Novel aspects:
We applied label free quantification to discover biomarker for the development of therapeutic agent in atopic dermatitis.

Abstract:
Quantitative proteomics measures abundance changes of many proteins among multiple samples in a high-throughput manner. Results from such measurements provide information on how biological systems respond to environmental perturbations at a genomic scale. A number of methods have been developed for quantitative proteomics to obtain high proteome coverage, accurate quantification, and wide applicability to different types of samples. Here, we used normalized spectral index based label free method for protein quantification. Label-free quantification can be achieved by correlating protein abundance with mass spectrometric signal intensities of peptides and the number of MS/MS spectra matched to peptides and proteins. In terms of accuracy, precision, and reproducibility, quantification performance was tested by using standard proteins samples. We compared normalized spectral index based label free quantitation with MS chromatogram based label free quantification. We applied this method to discover biomarker for the development of therapeutic agent in atopic dermatitis.

PMo-120
13:30 – 14:40

Multidimensional LC-MALDI Workflow for High Sensitivity Detection of Low-Abundance Peptides from Complex Samples

Shinichi Iwamoto[1], Yusaku Hioki[1], Ritsuko Tanimura[1], Yuki Ohta[1], Takashi Shimada[2], Koichi Tanaka[1]

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Keywords:
Multidimiensional LC-MALDI, Spotting robot, Column-integrated probe, PTM analysis

Novel aspects:
A novel spotting robot for the MALDI target was developed for the high sensitivity nanoflow LC-MALDI analyses and applied to the off-line multidimensional LC-MALDI workflow.

Abstract:

Introduction:
The discovery of protein biomarkers is important for early detection and elucidation of disease diagnosis. However, it is not easy to detect extremely small quantity of peptides from highly complex samples such as serum or plasma without effective fractionation and enrichment strategies. A proteomic strategy based on multi-dimensional liquid chromatography (MDLC) combined with mass spectrometric analysis is essential approach for the effective detection of such kind of low-abundance biomarkers. The purpose of our study is to construct an extremely high sensitivity MDLC and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) workflow available as a proteomics platform for the discovery of protein biomarkers and post-translational modifications (PTM) . Recently, we developed a prototype of a spotting robot on MALDI target, which is suitable for the high sensitivity nanoflow LC-MALDI analyses. In this presentation, we demonstrate and discuss the advantages of the off-line MDLC-MALDI workflow.

Methods:
The prototype of the novel MALDI spotter instrument was designed for the highly accurate collection of nanoliter fractions eluting from nano-LC system on MALDI targets to support 1536 well format. This spotter is equipped with a laser sensor for high-precision z-axis positioning with an overall error of 15 mm. The probes were constructed from 0.050 mm i.d. 0.150 mm o.d. x 150 mm length fused silica capillary tubing. *Cadenza*™ CD-C18 resin (Imtakt, Japan) was self-packed into the capillary with sol-gel frit. The 2,5-dihydroxybenzoic acid (DHB) precoated targets were prepared using piezoelectric inkjet device. MALDI-QIT-TOF MS (*AXIMA Resonance*™; Shimadzu/Kratos, UK) measurement was performed in positive ion mode.

Results:
The newly designed MALDI spotter is fully compatible with nano-LC system. The outside of column probe tip coated with a hydrophobic material allows various kinds of LC eluents including an acetonitrile gradient to deposit stably on a MALDI target. The performance of MDLC-MALDI system has also been evaluated in terms of a high selectivity and sensitivity detection of the PTM-containing peptides. To optimize peptides separation, hydrophilic interaction chromatography (HILIC) mode was used because of its orthogonality to 3-dimensional-reverse phase mode. The ion-exchange phase was also used to separate other sets of molecules which have similar hydrophobic/hydrophilic properties. The final column eluents were directly spotted on DHB precoated targets from capillary column-integrated probe using the nanoliter spotting robot to prevent post-column diffusion. The peptides with PTMs spiked in highly complex sample were reproducibly detected using our MDLC-MALDI system. The combination of MALDI-MS and MDLC is a powerful approach for the highly sensitive analysis of low-abundance peptides and therefore this is an effective breakthrough in the sample complexity in the field of proteomics or PTM analysis.

PMo-121
11:10 – 12:20

LC-ESI/MS analysis of protein modifications derived from a mixture of 13-HPODE and [13C18]-13-HPODE

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Keywords:
protein, modification, lipid, hydroperoxide, LC-ESI/MS

Novel aspects:
We have employed a mixture (1 : 1) of 13-HPODE and [13C18] -13-HPODE to facilitate the detection and characterization of protein modifications by LC-ESI/MS.

Abstract:
[Objective] Many studies have implicated oxidative stress as a substantial contributor to the degenerative diseases of aging, cancer, and cardiovascular diseases. Oxidative stress can induce the production of reactive oxygen species (ROS) and the activities of cyclooxygenases and lipoxygenases. They can oxidize polyunsaturated fatty acids (PUFAs) to form lipid hydroperoxides that undergo decomposition to highly reactive bifunctional electrophiles such as 4-oxo-2 (*E*) -nonenal (ONE) and 4-hydroxy-2 (*E*) -nonenal (HNE) . These aldehydes are involved in the inflammation, apoptosis and other cellular signaling through modification and cross-linking of the related proteins. Because of the difficulties in dealing with complex systems, most previous studies have focused primarily on either protein modification by a single electrophile or structural characterization of specific modifications on a model amino acid/peptide. It is now important to conduct a comprehensive study of the protein modifications results from various oxidation products of PUFAs. To develop the methodology of the global screening for lipid hydroperoxide-derived protein modifications, we have employed angiotensin II (Ang II : DRVYIHPF) as a model peptide. It is a well-known bioactive peptide in the renin/angiotensin system and has been found to be readily modified by reactive aldehydes such as ONE and HNE [1]. As for the source of electrophiles, 13-hydroperoxy- (*Z*,*E*) -9,11-octadecadienoic acid (13-HPODE) was prepared from the linoleic acid (LA) , the most abundant PUFA in mammalian tissue. [$^{13}C_{18}$] -13-HPODE was also prepared and a mixture (1 : 1) of 13-HPODE and [$^{13}C_{18}$] -13-HPODE was reacted with Ang II to facilitate the detection and characterization of Ang II modifications by liquid chromatography (LC) -electrospray ionization (ESI) /mass spectrometry (MS) . Thus, any modified Ang II derived from 13-HPODEs will be observed as a doublet (i.e. *m* : *m*+9 for ONE- or HNE-derived modification) in the mass spectrum with identical retention times by LC-MS. This will allow to develop the pseudo neutral loss scan method for an effective screening.

[Experiments] 13-HPODE and [$^{13}C_{18}$] -13-HPODE were prepared using soybean lipoxidase from linoleic acid and [^{13}C] -linoleic acid, respectively. 13-HPODE and Ang II were reacted in the presence of ascorbic acid (AA) or AA/Cu^{II} and analyzed to optimize the LC/MS condition. A mixture (1 : 1) of 13-HPODE and [$^{13}C_{18}$] -13-HPODE was then reacted with Ang II in the presence of AA or AA/Cu^{II} and analyzed using an optimized LC-ESI/MS conditions. LCQ-DECA (Thermo Scientific Inc.) equipped with ESI source was employed for the analysis of the reaction mixture. The chromatographic separation of reaction products was carried out on a SunFire C18 column (150 × 2.1 mm i.d., 3.5 μm ; Waters, Milford, MA) with water and acetonitrile containing 0.1% formic acid as mobile phases at 0.2 mL/min.

[Results & Discussion] The reaction conditions to generate 13-HPODE and [$^{13}C_{18}$] -13-HPODE from linoleic acid and [^{13}C] -linoleic acid, respectively, were optimized. They were isolated using normal phase HPLC system, and each concentration was determined. The oxidative condition for the reaction between 13-HPODE and Ang II was then optimized. The LC/MS condition was also optimized. It was revealed that each electrophiles-modified Ang II and the corresponding [$^{13}C_{18}$] -labeled one elute at the same retention time. The modified AngIIs derived from 13-HPODEs were confirmed by doublets in their mass spectra. The comprehensive pseudo neutral loss scan method will then be developed based on the current results for global screening of protein targets of electrophiles.

[1] Lee, S. H., Takahashi, R.,Goto, T., and Oe, T., *Chem. Res. Toxicol.* **23**, 1771-1785 (2010) .

PMo-122
13:30 – 14:40

Systematic investigation of peptide fragmentation processes in HCD on an Orbitrap instrument using a computer Expert System

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Max-Planck-Insitute of Biochemistry

Keywords:
automated MS/MS annotation peptide fragmentation

Novel aspects:
Peptide fragmentation knowledge can be successfully applied to large-scale datasets in an automated manner providing a unique insight into the composition of collision induced fragmentation spectra.

Abstract:
MS-based Proteomics projects often produce millions of MS/MS spectra containing information about tens of thousands of sequence unique peptides and their modifications. Peptide and protein identification based on database searches are largely automated and obtaining the peptide sequence from regular fragment ions seems straightforward. While the investigation of different peptide fragmentation mechanism has been of great interest to the mass spectrometric community for decades, this knowledge has not been applied to large-scale studies and it is difficult to access for biological researchers using proteomics as a tool. The latest generation of mass spectrometers that provides high resolution and high mass accuracy for both MS and MS/MS scans ('high-high-strategy') allows measuring the peaks in MS/MS spectra with high mass accuracy - for low m/z even revealing the chemical compositions. Here we develop a computer-assisted Expert System that incorporates much of the knowledge about peptide fragmentation pathways and we validate it by applying it to a statistical investigation into the nature of higher energy collisional dissociation (HCD) and collision induced dissociation (CID) fragmentat ion spectra.
We acquired data from digested E.coli, yeast and HeLa cell lysate on LTQ Orbitrap Velos and LTQ Orbitrap Elite mass spectrometers (Thermo Scientific) . Peptide identification was carried out with MaxQuant and the Andromeda search engine with a strict FDR of 0.0001. Our dataset initially contained more than 100,000 high scoring and unique tryptic peptides but was further filtered to obtain greater than 16,000 very high-quality MS/MS spectra nearly free of co-eluting peaks. The MS/MS spectra were then comprehensively annotated by an Expert System that was iteratively developed together with this study.
The major ion types that we found in our study can be classified into 6 different categories : regular ions that result from cleavage of the peptide back bone (a-, b- and y-type ions) , neutral loses, internal fragments, immonium ions, side chain fragments and the intact precursor mass. Strikingly, we find the amino acid coverage of our collection of tryptic peptides to reach 80% for a peptide length of up to 20 amino acid in total. This demonstrates high fragmentation efficiency and the potential for de novo sequencing. While HCD spectra in general feature highly complete y-ion series but only low mass b-ion series, in CID spectra the b-ion series also contributes substantially to the sequence information content. In contrast, there are many internal ions in HCD spectra (10% of the intensity coverage) , but these are nearly absent in CID spectra. Likewise, in HCD spectra 6 % of the intensity coverage is accounted for by immonium ions and side chain fragments are also specific and prominent in the low mass region. Our study demonstrates that peptide fragmentation knowledge can be successfully applied to large-scale datasets by our computer-assisted Expert System at a level of quality comparable or better than a human expert. This allows the expert focussing on very specific problems such as the discovery of novel fragmentation pathways.

PMo-123
11:10 – 12:20

Intraocular Lens Adsorbome: a Proteomic Study of Adsorbed Proteins onto Acrylic Materials and Its Implication in Secondary Cataract

Yi-Shiang Huang[1,2], Virginie Bertrand[1], Gabriel Mazzucchelli[1], Dimitriya Bozukova[3], Christophe Pagnoulle[3], Marie-Christine Durrieu[2], Edwin De Pauw[1], Marie-Claire De Pauw[1]

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Keywords:
Intraocular lens, Acrylic hydrophobic, Acrylic hydrophilic, Protein adsorption, Posterior Capsular Opacification

Novel aspects:
We illustrate the relationship between the absorbed proteins and the developement of secondary cataract. This finding will help scientists to design a new intraocular lens with lower secondary cataract rate.

Abstract:
The intraocular lens (IOL) is a polymer implant designed to replace the natural lens after cataract surgery. When the implant is introduced into the lens capsule, the polymer starts to interact with the aqueous humour and the exchange of molecules between the solid and the liquid begins. The nature of exchange in water, ions, and biomolecules may result in several postoperative complications including glistening, calcification, and posterior capsular opacification. The posterior capsular opacification (PCO, also called "Secondary Cataract ") is raised from the over-growth of residual lens epithelial cells. The first step of the over-growth process of the cells is their adhesion to the deposited biomolecules, such as proteins involved in extra-cellular matrices.
The purpose of this study is to identify the principal proteins adsorbed onto the acrylic polymers by mass spectrometry. The concept of adsorbome is to generate a list of adsorbed proteins to the hydrophilic and hydrophobic polymers, and then compare the difference to the original component of aqueous humour in order to see the affinity of individual protein to each material. Two kinds of hydrophilic and two kinds of hydrophobic acrylic polymers were tested for their adsorbomes by treating them with an aqueous humour analogue and the major adsorbed proteins were identified by mass spectrometry. Interestingly, the hydrophilic acrylic polymer shows a relative lower protein adsorption rate but shows a higher incidence of secondary cataract. This phenomenon implies the adsorbed proteins play a crucial role in progress of secondary cataract.

PMo-124
13:30 – 14:40

Analysis of SOD in cell line HepG2 by LC-nanospray-MS for screening natural antioxidants

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Keywords:
LC-nanospray-MS, proteotypic peptide, absolutely quantity, screening antioxidant activity

Novel aspects:
A cheap, low dose and label-free absolutely quantitation by LC-nanospray-MS is established and tested for CuZn-SOD in HepG2.

Abstract:
Generally, artificial free radicals, DPPH or ABTS are employed *in vitro* to screening antioxidant activity in natural resources. However, it is hard to infer directly the obtained free radical scavengers, neither the metabolites of the scavengers possessing antioxidant activity *in vivo*. Therefore, that is why bio-guided screening approaches are still in use, although they are cost and high dose of testing sample needed. In the present study, we developed an efficient bio-guide assay by detecting CuZn-SOD in HepG 2 using LC-MS for screening antioxidants. The novel aspect of study is cheap, low dose and label-free absolutely quantitation . We adopted trypsin to hydrolyze CuZn-SOD of HepG 2 and found good ion efficiency peptide sequences by LC-nanospray-MS. It is simply the recognition of the fact that not all of the peptides are equally likely to be detected by current mass spectrometry-based techniques. Therefore, we selected proteotypic peptide that is always detected and its analogue to synthesize. The analogue peptide sequence has been replaced one amino acid to anticipate that have similar ion efficiency to proteotypic peptide. These two synthesized peptides were used to acquire the absolutely quantity of CuZn-SOD in HepG2. And the CuZn-SOD in HepG 2 was in different treated by resveratrol. The quantity of CuZn-SOD was evaluated by the induced resveratrol . Nevertheless, if quantity of resveratrol adding in HepG 2 was too high, the CuZn-SOD containing in cell line HepG 2 would have decreased. Furthermore, the technique should be applicable for screening widely natural antioxidants.

PMo-125
11:10 – 12:20

Comparing and validating protein expression in multiple experimental conditions of antiviral immune response using clustering techniques on label-free LC-MS/MS data

Aivett Bilbao[1,2], Ying Zhang[1], Dario Bottinelli[3], Bandar Alghanem[1], Frederic Nikitin[2], Markus Mueller[2], Frederique Lisacek[2], Jeremy Luban[3], Caterina Strambio De Castillia[3], Emmanuel Varesio[1], Gerard Hopfgartner[1]

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Keywords:
LC-MS/MS, bioinformatics, label-free quantification, data quality control, analytical proteomics

Novel aspects:
Development of an alternative data quality control method to compare and validate protein expression across multiple experimental conditions

Abstract:
Classical vaccination approaches capable of controlling viruses have not worked for HIV-1. Several studies recently published indicate that mammalian cells have response mechanisms to HIV-1 through activation of innate sensors. The central coordinators of innate and acquired immune responses are the dendritic cells (DCs) and little is known about how they can detect retroviruses and help mounting an effective protective immunity against HIV-1.
A study applying a system biology approach capable of exhaustively exploring the protein landscape and the complexity of the innate immune recognition pathways in the DCs represents an opportunity to unravel the mechanisms by which these cells determine disease outcome and may provide important insights for the design of HIV therapies and vaccines.
Mass spectrometry has turned into the analytical technique widely used for data collection in proteomics research. At the same time, bioinformatics supplies computational methods for protein identification and quantification in these large-scale experimental data sets.
In this project, primary human DCs generated from peripheral blood monocytes of individual healthy donors (monocyte-derived DCs or moDCs) have been analyzed using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) instrumentation. These cells either elaborate a potent, innate, antiviral state that suppresses HIV-1 infection to undetectable levels or they are rendered completely permissive for HIV-1 infection.
As an initial screening, we are applying the bottom-up shotgun MS/MS workflow : after cell lysis of the moDCs samples, protein samples are digested and the label-free samples are analyzed using data-dependent (IDA) or data-independent (SWATH) acquisition schemes on a QqTOF mass spectrometer (with a resolving power of 30,000) . Then, standard peptide fragment fingerprinting (PFF) tools such as MASCOT and ProteinPilot are used for protein identification.
We are currently exploring approaches to compare and validate the protein expression level consistency across different donors and across different replicates (within the same donor) , as well as the protein expression level variations as a result of viral infection.
Preliminary results have shown some variability in the protein expression content among the multiple experimental conditions : however, to quantify and perform similarity analysis on these complex biological mixtures supporting the investigation of the antiviral response mechanisms is a difficult task.
To achieve this, we are developing a novel approach that consists in clustering the samples according to the different experimental conditions. Each sample is represented by a fixed-length vector where each dimension corresponds to a protein (from the list of identified proteins) and the value for the corresponding dimension is an estimated quantification. Different identification results and different label-free quantification strategies such as spectral count and MS1-based profiling (e.g. generated by SuperHirn) will be investigated using several clustering algorithms and similarity measures. In addition, we plan to extend the analysis to peptides and study post-translational modification profiles.
We expect the results to indicate whether the protein/peptide quantification content properly characterizes these multiple experimental conditions in order to : i) validate the protein expression level consistency intra and inter donors under the same condition (i.e. grouped in the same cluster) , and ii) identify possible distinctive patterns in each of the different conditions (i.e. samples under different conditions clearly separated in different clusters) .
The usage of such method as an alternative data quality control process is an important step in our project in order to reduce artefacts induced by individual variability and reliably categorizes the list of differentially expressed candidate proteins and signature peptides of the complex mixture under several basal and perturbed experimental contexts. Items of the output list will be further validated and characterized to create and refine models describing the biological pathways under study.

PMo-126
13:30 – 14:40

Identification of a modified amino acid residue in the heme protein using LC/MS/MS

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Keywords:
LC/MS/MS, peptide, triple quadrupore

Novel aspects:
Modified amino acid sequence identified using ultra fast LC/MS/MS scanning with multiple collision energy events.

Abstract:
An amino acid in a heme protein can be modified by reaction with hydrogen peroxide, resulting in loss of physiological activity of the protein. Additionally, it has been reported that the insertion of tryptophan near the heme in myoglobin can be oxidized by an organic acid peroxide.
In this study, we found that a methionine residue is modified in a mutant of another heme protein. However, among the peptide fragments obtained by the peptidase digestion of the modified protein, two peptides were present with the same molecular weight, containing methionine and constructed with the same set of amino acids. From this we identified the modified amino acid in the mutant protein using LC-MS/MS.
The mutant of the heme protein used in this study was a mixture of modified and non-modified proteins, with about 50% of the native protein oxidized. After digesting the mutant of the heme protein with trypsin, the obtained peptide fragments were analysed by mass spectrometry. The sample was analyzed using a double-focusing mass spectrometer with electrospray ionization. Then LC-MS/MS analysis was performed using UHPLC (Nexera, Shimadzu Corporation, Japan) and a triple quadruple mass spectrometer (LCMS-8030, Shimadzu Corporation, Japan) . Separation was achieved using an ODS column, Shim-pack XR-ODS II (150 x 2.0 mm, 2.2um) . The sample was eluted at 0.2 mL/min with a binary gradient system and analysed by MS/MS in positive ion electrospray.
The tryptic digested sample of the heme protein was analyzed by ESI-MS. Peptide F 2 (sequence : IFIMK) and peptide F 8 (sequence : MIFIK) at m/z 651 (M+H) differed in sequence. Peak at m/z 667, which was 16u larger than the original peak is likely to be due to the oxidized species of peptide F 2 or F8. MS/MS analysis of two products at m/z 326 (M+ 2 H) corresponded to the mass of the peptides F 2 or F8, and the peak at m/z 334 (M+ 2 H) to the modified peptide. In order to analyze the sequence of the peptide exhibiting these peaks, product ion scan (MS/MS) measurements were performed with three collision energies (CE=10, 20, 30V) and a high scan rate (15000u/sec) . The results of product ion scan data showed that the two peaks at m/z 326 were F 2 and F8, respectively, and that the peak of m/z 334 was F 2 (IFIMK) .

PMo-127
11:10 – 12:20

Population proteogenomics: qualitative and quantitative analysis of SNP and protein markers for obesity from plasma proteome

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Keywords:
single nucleotide polymorphism, SNP, multiple reaction monitoring (MRM) , obesity

Novel aspects:
We developed proteomics pipeline to analyze genetic variations in the proteome level analysis for population scale.

Abstract:
With the completion of the Human Genome Project, more than 3 billion bases in the human genome were fully sequenced. Among the human genome, 99.9 % of bases are exactly the same from person to person. Approximately 3 million bases in the human genome are different, and these variations make people unique. Single nucleotide polymorphisms (SNPs) are the most common type of these genetic variations among people and the major genetic basis of physiological or pathological variants for individuals. There are two types of SNPs within coding region : synonymous and non-synonymous. The non-synonymous SNPs (nsSNPs) result in variation of amino acid sequences and often have significant impact on functions of the corresponding proteins and also phenotypes. However, little attention has been paid to study SNPs at the proteome level in population scale so far. Therefore, in this study, we present a new proteomic analysis pipeline based on high-confidence Uni-prot annotation and proteins that were analyzed by shotgun proteomics strategy. In this study, we applied a targeted proteomic approach, multiple reaction monitoring (MRM) , in total 300 plasma samples to quantitatively analyze and find associations between nsSNP and obesity at the proteome level in population scale.

PMo-128
13:30 – 14:40

Optimization of chemical derivatization of nitro-peptides for fluorous solid-phase extraction.

Ji Hye Hong, Kyung-Cho Cho, Jeong Won Kang, Kwang Pyo Kim

Konkuk University, Seoul, Korea

Keywords:
nitration, Post-translational modification, Mass spectrometry,

Novel aspects:
To enhance enrichment efficiency of nitro-peptides, we optimized the blocking step of free amino groups on the nitro-peptides.

Abstract:
Protein tyrosine nitration is nitric oxide (NO·) dependant post-translational modification (PTM) associated with a number of diseases and biological aging. To correlate protein tyrosine nitration mechanistically with specific physiological and pathological conditions, it is important to identify protein target sequences and to quantify protein-3-NT residues on these proteins. Since protein nitration is a low-abundant post-translational modification, development of an effective enrichment method is needed to detect nitrated peptides or proteins from the limited amount of patho-physiological samples. The developed enrichment method includes blocking, reduction, and specific chemical tagging steps of the nitro-peptide prior to capturing the derivatives with fluorine carbon tag. However, blocking by acetylation resulted the reduction of mass signal. As the blocked peptides with acetylated lysine on C- terminal have positive charge at amine group, they are induced to be neutralized during ionization in ESI-MS. Thus, the choice of acetic anhydride as an acetylating agent results in fatal reduction of peptide ion signals during ionization in MS. To overcome the problem associated with signal reduction due to acetylation, in this study we introduced dimethylation step to block amino groups on tryptic peptides keeping their positive charge after the blocking reaction.
At first, we synthesized two artificial peptides with only differed C-terminal sequence. One had C-terminal lysine, the other had C-terminal arginine as a control. Then two peptides go through the same processes of blocking, acetylation and dimethylation, followed by analysis on ESI-MS. Our results indicate that the peptide with C-terminal lysine showed significant reduction of mass signal when used acetylation for blocking, however, we could show the mass signal gain when we use dimethylation as a blocking.
We conclude that the blocking step of tryptic digested peptides using dimethylation is more efficient than acetylation without loss of signal for ESI-MS analysis.

PMo-129
11:10 – 12:20

Quantification of biological drugs in plasma by liquid chromatography-tamdem mass spectrometry.

Nozomu Kato[1], Takesada Shimura[2], Atsuko Takeuchi[4], Hisahide Nishio[3]

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Keywords:
Quantification, biological drugs, LC-MS/MS

Novel aspects:
Quantification of biological drugs in plasma by liquid chromatography-tamdem mass spectrometry was performed.

Abstract:

[Objectives]

In many cases, enzyme-linked immunosorbent assay (ELISA) methods are developed to quantify the biological active proteins in plasma samples. However, in early stage of drug discovery, ELISA will not be available for candidate proteins because of not to be prepared yet the specific antibody for ELISA. Then the discussion on the relationship between PK and PD might be skipped, though it is very important to make go/no go-decision even in early stage. Our objective is to establish the method for quantifying the biological drugs in plasma and take PK information in animals, without a specific drug antibody, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) .

[Methods]

To indicate the usefulness of LC-MS/MS method, three types of biological drugs on market , Palivizumab as a whole IgG (Abbott) , Etanercept as a fusion protein of TNFalpha-Receptor and Fc fraction (Pfizer) , and Peginterferon as a PEGylated IFN-alpha-2b (MSD) , were examined. Firstly, a specific peptide, which was necessary as a probe peptide for each protein to be detectable by mass-spectrometry, was selected from a portion of protein sequences using available tools and data base on the web site (Expazy, NCBI or Swiss-prot) considering some defined conditions. The each specific peptide and its peptide with stable isotope (IS) for proteins were synthesized. Next the standard samples were prepared for calibration curve and QC. A specific peptide and IS were spiked in the tryptic digested plasma and samples were injected to LC-MS/MS (UPLC-Quattro Premier, Waters) to quantify the specific peptide. A validation (recovery, linearity of calibration curve, intra-day and inter-day assays) were performed. Finally, a single dose PK study in animals was carried out to examine the usefulness of LC-MS/MS method against ELISA method.

[Results and Discussion]

The validation results were satisfied our criteria for Palivizumab. This validated method was adapted to the PK studies of Palivizumab (rat and monkey) . In results, the PK parameters are good correlated good correlated to previous reported values determined by ELISA. For Etanercept the recovery % from standard samples was relative low, therefore Etanercept was added to plasma before tryptic digestion and digested in order to correct the recovery %. This protocol could provide results with good precision. The validation of Peginterferon is still under consideration.

PMo-130
13:30 – 14:40

Label-Free Semi-Quantitative Analysis of Histone Modification Dynamics using High-Resolution Mass Spectrometer

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Keywords:
LC-MS/MS ; Label-Free semi-Quantitation ; Histone Modifications ; ETD ; Cell Cycle

Novel aspects:
Improvement in sensitivity and resolution of proteomic analysis for histone modification dynamics.

Abstract:
Purpose : Improve methodology and workflow of histone modification analysis to identify function-dependent combinatorial histone codes.

Background : Histone modification has been an attractive target for MS. Especially, two types of histones, H3 and H4 have many modification sites (amino acid residues) which can be methylated (mono-, di-, tri-) , acetylated, phosphorylated, and more. These modifications can occur in any combination and produce thousands of possible patterns. Different modification pattern does not necessarily mean different mass or chemical property. Rather, the difference is often extremely subtle, so the good separation and the fast high-resolution mass measurement are essential. In addition, increasing interest in epigenetics demands more sensitive analysis. Histones carry rich epigenetic information in the form of posttranslational modifications on their N-terminal tail sequences, playing crucial roles in dynamic cellular function. Their combinations of modifications constitute the "histone code " which regulates epigenetic events.

Methods : Our approach is label-free semi-quantitative LC/MS/MS to investigate time-course changes in multiple modification patterns. For H4 analysis, histone was extracted from cell-cycle synchronized HeLa S3 cells, digested with Asp-N, and analyzed with nano LC-MS/MS (Orbitrap ELITE ETD) . We used Progenesis software (Nonlinear Dynamics) for quantitative analysis. Data were then subjected to multivariate analyses.

Results : With about 1 μg of histone proteins, we determined over 50 patterns of combination of modifications. Notably, the phosphorylation of serine in H4 (H4S1ph) was well-quantified, which has been otherwise poorly detected or quantified so far. In the cell-cycle synchronization experiments, we observed cell cycle phase-dependent ups and downs of H4S1 phosphorylation level with good reproducibility, as well as H4K20 methylation changes described previously.

Conclusion : We have developed an improved methodology and workflow to analyze histone modification dynamics.

PMo-131
11:10 – 12:20

Simultaneous quantification of CYP1A2, 2D6, and 3A4 isoform proteins by liquid chromatography-tamdem mass spectrometry using a single internal standard peptide.

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Keywords:
quantification, cytochrome P450, LC-MS, single internal standard peptide

Novel aspects:
Simultaneous quantification of CYP 1 A2, 2 D6, and 3 A 4 isoform proteins by liquid chromatography-tamdem mass spectrometry using a single internal standard peptide was performed.

Abstract:

[Objectives]

Nowadays, quantification of enzyme proteins by liquid chromatography-tamdem mass spectrometry (LC-MS/MS) is adopted widely in evaluation of drug-drug interaction caused by drug-metabolizing enzymes. Typically, in quantifying each cytochrome P450 (CYP) isoform protein, a stable isotope-labeled peptide specific to each CYP isoform protein is utilized as an internal standard (IS) . In this method, a stable isotope-labeled peptide has to be synthesized for each isoform to be measured, which constitutes a drawback. Therefore, we established a simultaneous quantification method using a single IS peptide for major three CYP (CYP 1 A2, 2 D6, 3 A 4) isoform proteins, which are important in the incipient period of drug discovery.

[Methods]

Probe peptides of CYP 1 A2, 2 D6, and 3 A 4 isoforms were selected from analysis data of Matrix Assisted Laser Desorption/ Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) and literature information. A peptide that does not exist *in vivo* was used as a single IS peptide. By using these peptides, we measured content of each CYP isoform protein in individual (Lot HG3, HG6, HG23, HG43, HG56, HG66, HG89, BD Gentest) or pooled human liver (Mix gender, Pool of 15, Lot PR09004, Xenotech) and intestinal microsomes (Mixed sex human, Pool of 6, Lot UGU, BD Gentest) as well as recombinant human (cDNA-expressed) CYP isoform proteins (CYP 1 A 2 Lot 456203, CYP 2 D 6 Lot 456217, CYP 3 A 4 Lot 456202 : baculovirus-expressed microsome, BD Gentest and CYP 1 A 2 Lot C 1 A 2 R007/A, CYP 2 D 6 Lot C 2 D 6 R013/A, CYP 3 A 4 Lot C 3 A 4 R010/A : *Escherichia coli* expressed microsome, Cypex) . We also measured content of CYP isoform proteins in cryopreserved human hepatocytes treated with rifampicin. These quantitative results were compared with those measured with a stable isotope-labeled peptide IS specific to each CYP isoform and validity of the measurement method that we established was verified.

[Results and discussion]

Contents of CYP 1 A2, 2 D6, and 3 A 4 isoform proteins in liver microsomes of each donor obtained with the measurement method using the single IS peptide were almost identical to those results using the stable isotope-labeled peptide ISs for each CYP isoform. Moreover, the measurement results in intestinal microsomes and recombinant human CYP microsomes with a different matrix in which a CYP protein exists were almost the same. Furthermore, expression induction of CYP 3 A 4 isoform alone was confirmed when contents of each CYP isoform protein after rifampicin (a CYP 3 A typical inducer) treatment using the cryopreserved human hepatocytes were quantified. Thus, the assay that we established is one of an effective tool in drug discovery because contents of multiple CYP isoform proteins are measurable with the single IS protein.

PMo-132 Testing of LC-MS/MS identification criteria for ricin protein

13:30 – 14:40

Martin T Söderström, Paula Vanninen

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Keywords:
ricin, identification criteria

Novel aspects:
Establishment and testing of well-defined criteria for target protein identification in an forensic-type analysis.

Abstract:
The Chemical Weapons Convention (CWC), which entered into force in 1997, prohibits the development, production, acquisition, stockpiling, retention, transfer or use of chemical weapons by Member States. The Organisation for the Prohibition of Chemical Weapons (OPCW) has been established to implement the CWC. Compared to other treaties limiting weapons of mass destruction, the CWC aims at complete elimination of this type of weapons. It also includes a verification aspect in the form of routine and possible challenge inspections as well as investigations of alleged use of chemical weapons.

Typical analytes covered by the CWC are small molecules, which can quite routinely be analysed by GC-EI/MS, LC-ESI/MS or LC-APCI/MS. Ricin is the only protein toxin included in the CWC. As a minimum, the identification by two different analytical techniques is required. These identification criteria are not directly applicable to a much more complex system as the ricin protein. The Scientific Advisory Board (SAB) of the OPCW and its temporary working group has been discussing the establishment of identification criteria for ricin.

Ricin protein is found in seeds of *Ricinus communis*, the castor plant. It is a ribosome inactivating protein (RIP), which consists of A and B chains (30 kDa and 29 kDa without glycosylation). The mass of the glycosylated protein is 63 kDa. The B chain is responsible for allowing the toxin to enter cells while A chain is RIP active. The seeds also contains *Ricinus communis* agglutinin (RCA) which consists of two A and two B chains and has weight of 120 kDa. RCA is closely related to ricin and these two proteins contain common sequence fragments.

Typically LC-MS/MS identifications of proteins are based on database searching. The analysis is done from an enzymatically digested sample. In these analyses, the product ion spectra are measured using either clearly defined or data-dependent measurements. The data, full raw data or pre-processed data, are searched in protein databases. Finally, the resulting peptide matches are correlated with possible proteins present in the samples. The reliability of result is increased with increasing sequence coverage.

The identification of ricin for the purposes of the CWC has several requirements: 1) the presence of both A and B chains have to be verified, 2) it has to be verified that the chains are connected and 3) the ricin protein is RIP active. The traditional identification method described above could fulfil the two first requirements. In our studies, we have chosen a more direct way also able to fulfil these two requirements for the identification, which aims for high sensitivity. The third requirement can be satisfied only with activity assays, which are outside the scope of this paper.

The starting point for our work has been the analysis of the sequences of both ricin and RCA. The aim has been to find tryptic peptides, which can be used to differentiate ricin and RCA as well as be as characteristic to ricin as possible. The analysis of ricin samples has been carried out by a triple quadruple LC-ESI/MS/MS instrument using both product ion scan and selected reaction monitoring (SRM). The basic identification criteria used for the evaluation of this data include comparison retention time information as well spectral or SRM data between sample and reference. For protein identification, the number and properties of the peptides as well as the relative intensities seleted to represent the protein play an important role. The detection limits depend greatly on the selected criteria.

PMo-133
11:10 – 12:20

The Precision of Heavy-Light Peptide Ratios measured by MALDI-TOF Mass Spectrometry and its Application to LC-Free SISCAPA Protein Quantification

Detlev Suckau[1], Leigh Anderson[2], Morteza Razavi[2], Terry Pearson[2], Rainer Paape[1]

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Keywords:
peptide, quantitation, MALDI, SISCAPA

Novel aspects:
Peptide quantitation using stable isotope standards and MALDI-TOF MS yields accurate results with CVs in the 1 - 2 % range from plasma.

Abstract:
The most critical step in translating candidate protein markers into clinical diagnostics, the so-called verification step, requires accurate protein and peptide quantitation in large sample sets (usually plasma or serum) . Nano-LC and multiple reaction monitoring (MRM) mass spectrometry have been methods of choice providing precise relative quantitation of proteotypic signature peptides representing proteins of interest in clinical samples. However, the use of nanoLC inhibits use of this approach in clinical settings. SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies) are introduced and combined with MALDI-TOF MS as an LC-free method for high throughput protein quantitation. MALDI-TOF is characterized as an extremely simple and precise assay methodology that may be applicable for measurement of diagnostic proteins in clinical laboratory environments.
Six pairs of synthetic proteotypic peptides were used in 2 forms : unlabeled (L) and labeled (H) in the C-terminal K or R residue. Peptides were proteotypic for human proteins such as thyroglobulin, PCI, HER 2 /neu, HE-4, amongst others. Peptides were used from 10 mM stocks to prepare mixtures at varying L : H ratios across 11-point calibration series spanning ranges up to 2047 fold. Dilutions were prepared in 96 well plates using a liquid-handling robot (Bravo, Agilent) and spotted on prespotted AnchorChip MALDI plastic targets (Bruker Daltonik) in quadruplicate. The SISCAPA assays utilized Invitrogen ´ s protein G dynabeads, rabbit monoclonal antibodies selected for high peptide affinity and human plasma with titrated levels of spiked peptides. MALDI-MS spectra were acquired on an autoflex speed LRF MALDI-TOF in positive ion mode.
The precision of peptide quantitation by MALDI-TOF MS was explored using 6 pairs of proteotypic peptides (L) and same-sequence stable isotope labeled synthetic internal standards (H) . These were combined in dilution curves spanning up to 2,047-fold ratios. Coefficients of variation (CV) of L : H peak area ratios were examined across 4 replicate MALDI spots per sample.. Averaged across 11 points of a 100-fold dilution curve and over all 6 peptides, the overall CV was 2.5% at 11 fmol total (light + heavy) of each peptide applied per spot. The average CV of measurements at near-equivalence (the center of the dilution curve) for the six peptides was 1.0%. Response curves across the 100-fold range could be closely modeled by a power law fit giving R^2 values > 0.998 for all peptides.
The MALDI-TOF MS method was used to determine the endogenous level of a proteotypic peptide (EDQYHYLLDR) of human protein C inhibitor (PCI) in a plasma digest after enrichment by capture on a high affinity anti-peptide antibody, a technique called Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) . The level of PCI was determined to be 770 ng/mL with a replicate measurement CV of 1.5% and a >14,000-fold target enrichment via SISCAPA-MALDI-TOF. These results indicate that MALDI-TOF technology can provide precise quantitation of high-to medium abundance peptide biomarkers over a 100-fold dynamic range when ratioed to same-sequence labeled internal standards and enriched to near purity by specific antibody capture. The robustness and throughput of MALDI-TOF in comparison to conventional nano-LC-MS technology could enable currently impractical large-scale verification studies of protein biomarkers.

PMo-134 Production of Standards for Quantitative Proteomics and Phosphoproteomics with Focus on ICP-MS and ESI-MS

13:30 – 14:40

Wolf D Lehmann

German Cancer Research Center (DKFZ)

Keywords:
quantitative proteomics, peptide standards, protein standards, phosphorus, selenium,

Novel aspects:
Production of peptide/protein standards calibrated via ICP-MS and phosphorus or selenium detection and their transformation into phosphorus-free or selenium-free peptide/protein standards under preservation of their accurate quantification.

Abstract:
Quantitative data are of outstanding importance in the life sciences. Regular quantifications result in concentration data. Quantitative proteomics started with relative quantifications, designed for measurement of concentration ratios. In general, relative quantification methods are connected with differential analyte labeling, so analytes from samples to be compared can be distinguished when analyzed as mixture. Later, methods for regular quantification ("absolute quantification ") followed, which deliver concentration data. For this purpose, the method of choice is to use a standard which is both labeled and calibrated. For further development of the field of quantitative proteomics, there is urgent demand for accurately quantified and certified peptide and protein standards. Techniques capable for generation of such standards are described with focus on inductively-coupled plasma ionization mass spectrometry (ICP-MS) and electrospray ionization mass spectrometry (ESI-MS) as quantification methods.

For quantification of phosphopeptides by ICP-MS, phosphorus is the element of choice. (PASTA peptides [1]) . Using quantitative dephosphorylation controlled by ESI-MS or LC-ESI-MS, quantified phosphopeptide standard solutions can be converted into peptide standard solutions, with preservation of the quantification accuracy. In this way it is possible to produce phosphorus-quantified but phosphorus-free stable isotope labeled peptide standards. Mixing of calibrated peptide and phosphopeptide solutions results in ratio standards, which serve e.g. as standard for determination of a site-specific phosphorylation status [2] . Ratio standards work without quantification, so that the error of the adjusted ratio is only connected with a volumetric mixing error.

For specific quantification of intact proteins by ICP-MS, selenium is the element of choice. This concept is based on the production of selenomethionine (SeMet) labeled proteins by cell-free protein synthesis. Recently, stable isotope (^{13}C, ^{15}N) and SeMet labeled proteins were produced by cell-free synthesis and quantified by ICP-MS and selenium detection (RISQ, [3]) . Another possibility is to introduce these two types of label into protein analogs expressed separately. The SeMet-containing protein is quantified by ICP-MS and then used as standard for quantification of its stable isotope labeled analog. In this way it is possible to produce SeMet-quantified but SeMet-free stable isotope labeled protein standards. The necessary extra quantification step can be performed with a relative standard deviation around 1 % [4] . Examples for protein quantifications and their calculated accuracies are given and discussed.

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PMo-135
11:10 – 12:20

Exploring the dynamic range of label-free mass spectrometric quantification in complex samples

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Keywords:
Label-free proteomics, dynamic range on UHR-TOF, quantitation in low fmol range

Novel aspects:
The in-depth determination of the dynamic range of label-free proteomic studies in complex mixtures using a Q-TOF instrument.

Abstract:
Introduction:
The proteome is characterized by its high complexity and wide range of analyte concentrations which are constantly in flux due to developmental and stress-related processes. Thus, discovery proteomics approaches are required which are completely unbiased and cover the complete proteome.

Accurate quantification of differently expressed proteins is still challenging and relies heavily on a stable analytical platform. Due to advances in instrumentation it is possible to perform quantification without the need for labeling based on the comparison of ion intensities delivering quantitative information for a theoretically unlimited number of samples.
In this study we will investigate the quantitative performance of label-free approaches with a focus on dynamic range.

Methods:
Tryptic peptides were separated on a nano UHPLC system (U3000 RSLCnano, Dionex) using a 120min gradient and data for label-free quantification were acquired with an Ultrahigh Resolution (UHR) Q-TOF system (maXis impact, Bruker Daltonics) equipped with a Captive Spray ionization MS source. A proteomic standard of 48 proteins spanning a concentration range of 6 decades in total (UPS-2 standard, Sigma) was used to determine the dynamic range for label-free quantification. This standard was spiked at two different concentrations (250 fmol to .5 amol and 500 fmol to 5 amol) into a highly complex *E.coli* background (500ng) mimicking the complexity typically found in biological samples. The label-free quantitation workflow was based on signal intensities from LC-MS runs for quantification and data from LC-MS/MS runs were used for identification information.

Results:
The determination of the dynamic range of label-free quantification was performed for a standard protein mixture spiked at 2 different concentrations into a constant *E.coli* background. The experimental setup covered concentration ranges from 500fmol down to 2.5amol. Separation of tryptic peptides on a nano LC system coupled to UHR-TOF detection showed excellent retention time and signal intensity stability for five replicates per concentration, both being a prerequisite for label-free quantification studies. Quantitative information was obtained from MS signal intensities. Signals originating from the same peptide, e.g. different charge stages and isotopes were combined and used for quantification. Results show almost no variation for E.coli background, whereas regulation ratios were determined for the standard peptides spiked into the complex background. In total 27 proteins from the UPS 2 mixture were detected as significantly regulated ($p<0.05$) . Very accurate quantification was obtained down to the low fmol range. Results also show the label-free approach to be capable of providing the correct quantification of peptides in the amol range. The high dynamic range of this approach allows in-depth studies of quantitative changes in biological systems. Therefore the method will be applied to determine the effect of sodium butyrate treatment of Chinese hamster ovary cells.

PMo-136
13:30 – 14:40

Multiple Products Monitoring Using Q-Exactive Mass Spectrometer (Q-MpM)

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Keywords:
accurate target quantification, multiple products monitoring, Q-Exactive

Novel aspects:
Combination of MpM with Q Exactive MS (producing fast and accurate MS 2 spectra) is very compatible and promising for accurate target quantification with high dynamic range in high complex sample.

Abstract:
Mass spectrometry-based proteomics has greatly emerged as useful technology for biomarker discovery and target verification. Target verification from biological and clinical samples is still challenging upon high complexity and high dynamic ranges. Since an MS 2 -intensity based quantification shows higher sensitivity and specificity, selected reaction monitoring (SRM) and multiple reaction monitoring (MRM) has been widely used for target quantification on triple-quardrupole mass spectrometry. However, we previously reported a robust target peptide quantification method : multiple products monitoring (MpM) from full MS 2 scans produced by ion trap mass spectrometer (Journal of Proteome Research, 2009, 8, 3625-3632) . A new type of orbitrap analyzer with quadrupole mass filter (Q-Exactive) was recently developed. Q-Exactive mass spectrometer enables fast multiplexed single ion monitoring following full range of highly accurate MS 2 scans (12 scans/sec with R=17500) . We applied MpM approach to Q-Exactive mass spectrometer platform. Moreover, we tested our MpM method (quantitative accuracy of target peptide quantification) applying Q-Exactive multiplexed single ion monitoring with standard light and heavy peptides showing high dynamic range. Combination of MpM approach with Q-Exactive (Q-MpM) is very promising for accurate target quantification with high dynamic range in high complex sample.

PMo-137 In-Source Decay for the high-throughput sequencing of animal toxins

11:10 – 12:20

Loic Quinton[1], Michel Degueldre[1], Nicolas Gilles[2,3], Pierre Escoubas[3], Edwin De Pauw[1]

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Keywords:
venomics, toxins, ISD, MALDI

Novel aspects:
High-throughput sequencing by ISD

Abstract:
Animalvenoms are complex chemical cocktails, comprising a wide range of biologically active reticulated peptides that target with high selectivity and efficacy avariety of membrane receptors such as ion channels and G-Protein Coupled Receptors. Based on various disulfide-linked scaffolds, they represent anenormous structural and pharmacological diversity. Assuming the fact that each of the 170,000 venomous species reported can produce in their venom more than 250 bioactive, the global animal venom resource can be seen as a collection of more than 40,000,000 bioactive peptides and proteins. These toxins are continuously selected and highly refined by the evolution process, up to the point where every molecule is endowed with pharmacological properties that are highly valuable in the context of human use and drug development.

A FP7 European Project called VENOMICS (http：//venomics.eu/) was designed and started in last November. This project combine resources, skills and knowledge of the eight partners that represent an efficient mix of academic institutions (CEA (France) , University of Liège (Belgium) , Université de la Méditerranée (France)) and SMEs (VenomeTech (France) , Sistemas Genómicos (Spain) , NZYTech (Portugal) ,Zealand Pharma (Denmark) , Vitamib (France)) . The goal of this project is to construct a toxin bank of 10,000 sequences generating from the study of 200 selected venomous species. This bank will be exploited to discover new peptides that can be used as human drugs. This ambitious project imposes high-throughput strategies combining transcriptomic and proteomic sequencings, chemical and biochemical productions, and biological activity screenings. High-throughput proteomics of crude venoms represent one of the major bottlenecks of this project. Part or total peptidic sequences of toxins are hardly needed to allow the other partners to start their own tasks.

This work presents the strategy designed to generate high throughput accurate peptide sequences from crude venoms. The sequencing strategy rests on the chromatographic purification of venoms hyphenated with MALDI-In-Source Decay. ISD has already been demonstrated efficient for toxin sequencing [1] , and especially when using 1,5-DAN as reducing matrix [2] . ISD yields sequences covering more than 70% of the sequence by series of singly charged c-type ions even for high molecular mass toxins (> 6500 Da) . ISD spectra are simpler than classical MS/MS spectra and automation of spectra interpretation, difficult with other fragmentation techniques (CID, ETD) , is possible from ISD spectra. ISD requires however simple peptides mixtures that can be obtained using LC fractions collection on the MALDI place (LC-MALDI) . In addition 'in-house'software performed automated sequence analysis even when up to three peptides are within the same MALDI spot. [3]

Another part of our work is based on the use of Ion mobility (IMS) employed to control the quality of peptides produced by recombinant technology, with a special focus for the correct pairing of cysteins in disulfide bridges.

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PMo-138
13:30 – 14:40

Screening and Quantitative Analysis of Specific Proteins in Clinical Samples by LC-ESI-MS/MS

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Keywords:
Proteomics, Protein, LC-MS/MS, LTQ FT, QTRAP 5500

Novel aspects:
Specific biomarker proteins to disease were identified with combination of screening and quantitative analysis by LC-MS/MS using small amounts of clinical samples.

Abstract:
<Introduction>
To explore biomarkers for the identification specific diseases (e.g. cancer) , it's important to target the proper biomarker protein candidates and evaluate their expression including low level expression proteins from clinical samples, directly. Using clinical samples obtained from patients is one of the most effective ways to evaluate disease specific proteins because the identified proteins information can be linked to the disease state. One of the ways to evaluate if that the targeted proteins are disease specific, is immunoassay including ELISA or ECL. However, immunoassay requires an unique antibody and determines only one target. Therefore, as previous stage for immunoassay, it is beneficial to screen for and verify disease specific proteins from clinical samples by LC-MS/MS.

<Objective>
The objective of this study is to establish rapid and effective screening methods to identify positive biomarker protein candidates from the huge number of proteins in clinical samples and quantitative verification methods of the targeted proteins.

<Methods>
Non-metastasis tissues of breast cancer (subjects 6) and the metastasis tissues of breast cancer (subjects 19) were used from clinical samples. The cytoplasm was separated from each tissue specimen by centrifugation. The purified samples for each subject were alkylated with iodoacetamide and digested with trypsin. For the first screening process, the tryptic digested samples (4 μg total protein) were analyzed with 2D LC-LTQ FT. For the second screening process, the digested samples (0.4 μg total protein) were semi-quantitatively analyzed by multiple reaction monitoring (MRM) mode without internal standard using a QTRAP 5500, and the highest level peptides were selected as potential biomarker proteins. Finally, the tryptic digested samples (3 μg total protein) were quantitatively analyzed by MRM with stable isotope-labeled peptides as internal standards. From the results, expression of the targeted proteins was verified and evaluated the specificity to the metastasis group.

<Results>
During the first screening, 59 proteins were selected for analysis by 2D LC-LTQ FT. The second screening, by semi-quantitative MRM analysis, found four proteins that contained higher levels of certain peptides and were selected as biomarker specific proteins candidates. Finally during MRM analysis using stable isotope peptides as internal standards, two proteins were identified as biomarker specific proteins to metastasis of breast cancer. Furthermore, the most disease specific protein was also positively confirmed by quantitative RT-PCR.

<Conclusion>
Positive biomarker proteins specific to metastasis of breast cancer were selected efficiently from huge number of proteins at a low cost using a small number of clinical samples using LTQ FT. The selected proteins were identified and confirmed to be specific to metastasis of breast cancer at low cost using semi-quantitative and quantitative LC-MS/MS. Therefore, the combination of the screening methods and the quantitative verification methods is an effective way to identify the disease specific proteins using clinical samples.

PMo-139 Analysis of modified amyloid beta peptide by Mass Spectrometry

11:10 – 12:20

Akutsu Hiroaki, Tsumura Naomi, Funakoshi Hiroshi, Nakamura Masao

Asahikawa Medical University, Asahikawa Japan

Keywords:
Amyloid beta peptide, Tyr10, Met35, Asp1, Halogen

Novel aspects:
Here we present the different reaction of Amyloid beta with hypochlorous acid and hypoiodous acid.

Abstract:

Introduction

Accumulation of the amyloid beta peptide (Aβ) in the form of senile plaques (SPs) is a pathological hallmark of Alzheimer's disease (AD). Although the progression of AD is linked to inflammatory and oxidative processes, the biochemical mechanism underlying AD is uncertain. SPs consist of Aβ1-42 and Aβ1-41, and contain high concentrations of iron, copper and zinc. Recent findings show that myeloperoxidase (MPO) and microglia areco-localized with SPs. In addition to phagocytosis, microglia secrete proteases that degrade Aβ. In addition, microglia are activated by Aβ *in vivo* and generate H_2O_2.The interaction of Aβ with microglia is reported to stimulate the inflammatory process and may contribute to neuronal damage. We have reported the reaction of full length Aβ (Aβ1-42, Aβ1-40) and Aβ25-35 with hypochlorous acid [1]. The results indicate that Met35 in Aβ rapidly reacts with hypochlorous acid. In this study, we focused on the reaction of various Aβ containing no Met with MPO-derived oxidant, hypohalous acid and compared with the result of full length Aβ.

Method

Hypochlorous acid (HOCl) and hypoiodous acid (HOI) were formed by reaction of MPO in the present of H_2O_2 and KCl or KI. Reaction mixture containing 40μM Aβ1-12 was treated with MPO system. Reactions were carried out in 20mM phosphate buffer (pH 6). Mass spectra were observed by LC/MS/MS at indicated time after reaction was started. A NanoFrontier eLD LC/MS/MS system (Hitachi High-Tech) was used to obtain Mass spectra with MonoCap C18 Fast-flow (0.05 x 150 mm, GL Sciences). The mobile phase consisted of solvent A (2% acetonitrile with 0.1% formic acid) and B (98% acetonitrile with 0.1% formic acid). The acetonitrile gradient consisted of 15 to 40% at the flow rate of 200nL/min.

Results and Discussion

The mass spectrum of native Aβ1-12 showed a signal at *m/z* 712 ($[M+2H]^{2+}$) and 475 ($[M+3H]^{3+}$), respectively. When the Aβ1-12 was treated with HOCl, the spectra gave a 17Da (double charge ion) increase at *m/z* 729 (retention time 14.1min and 15.0min) and 22Da (double charge ion) loss at *m/z* 690 (retention time 14.6min). CID analysis revealed that the signal of *m/z* 729 is 35Da increase at b-ion and a-ion respectively (retention time 15.0min) and b10+34 ion (retention time 14.1min). A peak at retention time 14.1min corresponds to the chlorinated Aβ1-12 at Tyr10. A peak at 15.0min corresponds to the chlorinated one at terminal amine of Asp1. The signal of *m/z* 690 is 44Da loss at b-ion and a-ion, respectively. These results depict the decarboxylation of Asp1 [2]. When the Aβ1-12 was treated with HOI, the spectra gave a 63Da (double charge ion), 42Da (triple charge ion) at *m/z* 775, 517 (retention time 8.2min) and 126Da (double charge ion), 84Da (triple charge ion) at *m/z* 838, 559 (retention time 9.9min) increase of *m/z* 712, respectively. CID analysis confirmed that the signal of *m/z* 775 is b10+126 ion and a10+126 ion. The results indicate that the one iodine was added to Tyr10 and the signal of *m/z* 838 is a10+252 ion indicates that the two iodines were added to Tyr10.

There is a difference between the reaction mechanism of HOI and HOCl with Aβ1-12. Chlorination of Asp1 was followed by the decarboxylation of Asp1 through the reaction of Aβ1-12 with HOCl. Subsequently, chlorine transfer from Asp1 to Tyr10 was observed. In contrast, iodination of Tyr10 was readily observed without formation of Asp1 iodination. We also report the result of Aβ25-35 with HOCl for the comparison.

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PMo-140
13:30 – 14:40

The trial of lower background in using a nanoelectrospray (nano ES) interface

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Keywords:
nanoelectrospray LC/MS/MS, Proteomics, peptide

Novel aspects:
We have a prototype made by appending a filter to the cover of nanES interface. Using filter attached caver, we were able to reduce the low background.

Abstract:
Recently, nanoelectrospray LC/MS/MS was used for in the field of biological samples analysis should not be without. We analyzed a femtomole level biological sample, it became a problem to get a good results was that the low background.
Usually, nanoelectrospray (nanoES) interface was often attached to use in the open air space. Therefore, the influence of the establishment environment of the device was often taken. We tried to put the normal cover on the nanoES interface that we had been obtained to reduce the low background. Next, we had fabricated a cover which was wearing a filter to a part of the cover. We confirmed to be lower than background measurement was performed filter cover attached to the nanoES interface that was used only nomal cover.
It thinks that it becomes more possible by a background having fallen down for the measurement of the quantity without a low concentration biological sample.

PMo-141
11:10 – 12:20

Fully automated chip-based nanoelectrospray combined with electron transfer dissociation for high throughput top-down proteomics

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Keywords:
NanoMate, ETD/CID, top-down analysis, proteomics

Novel aspects:
NanoMate robot was for the first time combined with electron transfer dissociation and introduced in proteomics for peptide and bioconjugate analysis as well as for top-down protein fragmentation.

Abstract:
Collision-induced dissociation (CID) , previously the only available fragmentation technique on ion traps exhibits some limitation, among which, the low throughput and impossibility to characterize in details posttranslational modifications in terms of site (s) and structure. The recently implemented electron transfer dissociation (ETD) performed on advanced and versatile mass spectrometers is regarded as an important step with real perspectives for top-down proteomics. The last few years have witnessed the breakthrough of microfluidics for electrospray (ESI) , in which our group was intensively involved [1,2] , and their superior performance in terms of ionization efficiency, spray stability, analysis reproducibility, sensitivity and pace. Applied together, ETD and CID may significantly increase the sequence coverage and provide added confidence to peptide/protein identification [3,4] . In the present work, chip-based nanoESI performed on a NanoMate robot was combined for the first time with ETD and ETD/CID on a high capacity ion trap (HCT) MS to yield an analytical platform on which peptide sequencing and high throughput top-down protein analysis is feasible.
Mass spectrometry experiments were performed on a High Capacity Ion Trap Ultra mass spectrometer incorporating an ETD module with fluoranthene as anionic reagent. Fully automated chip-based nanoelectrospray was conducted on a NanoMate robot incorporating ESI 400 Chip technology.
NanoMate-ETDapproach was optimized and tested first for high throughput sequencing of a simple peptide. As the analyte the standard substance P neuropeptide containing 11 amino acids (MW 1347.63 Da) was used. This advanced protocol developed for Substance P was further applied to a complex functionalized synthetic peptide : gonadotropin-releasing hormone (GnRH) -III linked via amide bond to Daunorubicin (Dau) -GFLG, (MW 2257.32 Da) . NanoMate-ETD of the triply protonated form of GnRH-III (Dau-GFLG) produced sequencing of the peptide backbone and N-C_{alpha} cleavage of the NH-CO linkage between the peptide and Dau moiety, with the preservation of the labile glycosidic bond. The $[M+2H^+]^{2+}$ product ion corresponding to Dau at *m/z* 257.53 enhanced the further characterization of Dau moiety by a subsequent CID experiment using the alternate ETD/CID mode available on the HCT MS instrument. Furthermore, with the present setup, the first top-down protein analysis by ETD could be demonstrated on a medium-size protein. As a model substrate, horse apomyoglobin (MW 16.95 kDa) was chosen. In this experiment, of major importance is the molecule multiple protonation under chip-nanoESI conditions ; this process evidenced by the signals corresponding to high charge states, ranging from 14+ to 21+, is a fundamental prerequisite of a successful top-down fragmentation by ETD. For the experiment using $[M+16H^+]^{16+}$ion detected at *m/z* 1060.32 as the precursor, the fragmentation by ETD MS^2 could be successfully accomplished within only 30 s and with a sample consumption of only 12 fmols. ETD mass spectrum summed over the scans acquired for 30 s yielded a number of c- and z-type of product ions corresponding to 80 % coverage of apomyglobin sequence.
The obtained results on simple and functionalized peptides as well as on a medium size intact protein allow the possibility to accomplish peptide sequencing by ETD and top-down protein fragmentation in a high throughput regime. A significant reduction of the time and sample consumption required for reliable peptide/protein identification was also noticed. In view of NanoMate-ETD potential, extension to large proteins and proteins bearing posttranslational modifications are certainly planned and foreseen in the near future.

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PMo-142 Analysis of nitroproteome in S.cerevisiae using chemical approach

13:30 – 14:40

Jeong Won Kang[1], Na Young Lee[2], Kyung-Cho Cho[1], Sang-Hyun Park[2], Kwang Pyo Kim[1]

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Keywords:
Protein Tyrosine Nitration (PTN) , Nitroproteome, mass spectrometry, signal transduction

Novel aspects:
Proteomic scale study was performed to elucidate physiological functions of protein tyrosine nitration in mating signal transduction of S.cerevisiae.

Abstract:
Protein tyrosine nitration (PTN) is a post-translational modification occurring under the action of series of nitrating agents generated under oxidative stress. It results in the addition of a nitro group (NO_2) in *ortho* position to the phenolic hydroxyl group of tyrosine residue. The analysis of 3-nitrotyrosine are very important in biologically and clinically because PTN is affects in many pathological events such as various cancers, diabetes, neurodegenerative and age-related disorders. However, the functional and biochemical analysis of PTN has been hampered by the low-abundance of nitrated species and the lack of efficient enrichment methods. We described here a chemical approach to enrich nitrated peptides or proteins by incorporating specific tagging groups in the peptides through simple chemical transformations followed by mass spectrometric analysis, and also we apply this method to S. cerevisiae. The study of S.cerevisiae nitration is very important because S.cerevisiae is a most simple eukaryote. Therefore, we can indicate how nitration affects in vivo signal transduction or protein-protein interaction through the study of nitroproteome in S.cerevisiae. In this study, we try to identification of nitroproteins from S.cerevisiae, and detection of biochemical functions of nitroproteins on mating pathway from several over-expressed proteins involved mating signaling. Finally, we try to find motif of nitropeptides, and structural analysis.

PMo-143
11:10 – 12:20

Mass spectrometric and bioaffinity MS investigations of proteins involved in metabolic and age related diseases

Marius I Iurascu[1], Claudia Cozma[1], Michael Gross[2], David Clemmer[3], Michael Przybylski[1]

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Keywords:
Affinity-MS, IMS, Fabry, Alzheimer's, oligomers

Novel aspects:
Mass spectrometry, ion mobility spectroscopy and bioaffinity MS aplications on proteins involved in metabolic and age related diseases

Abstract:
New mass spectrometric (MS) methods have proven lately an increasing range of applications within the life science domain, from the rare diseases in newborns, like Fabry, to high level occurrence, age related disorders, like Alzheimer's. Fabry is a lysosomal storage disease (LSD) characterized by the deficiency of the enzyme alpha galactosidase A (a-GLA A) which leads to the accumulation of globotriaosylceramide in different organs, causing their failure. To understand the molecular working of the enzyme the affinity mass spectrometry was chosen and a recombinant human alpha-galactosidase A was used. A mouse monoclonal antibody to human alpha-galactosidase A was immobilized on a sepharose column and its affinity to the a-GLA A was investigated by in-gel tryptic digestion. For the epitope identification an epitope-excision experiment was performed. The a-GLA A was immobilized on the antibody column and proteolytically digested with trypsin. After the wash of the supernatant, the epitope was eluted with 0.1 % TFA in milliQ and identified by mass spectrometry. Alzheimer's disease (AD) is a progressive neurodegenerative disorder which affects an increasingly proportion of the elderly population. The hallmark of the disease is the accumulation of intercellular high molecular weight aggregates in the form of amyloid plaques and fibrils, although, the key neurotoxic moieties are lately suggested to be the Amyloid-beta (Aß) oligomers. Aß is a 39-43 aa peptide that is formed proteolytically from the large ß-amyloid transmembrane precursor protein (APP) and is highly prone to spontaneously aggregate. For the Aß-oligomers studies by (i) ion mobility spectroscopy (IMS) and (ii) mass spectrometric proteome analysis, Aß (1-40) was synthesized using solid phase peptide synthesis (SPPS), Fmoc chemistry, and the crude peptide purified by RP-HPLC [1]. The peptide was characterized by electrospray (ESI) ion trap mass spectrometry and by high resolution MALDI FT-ICR MS. After synthesis and purification, the Aß (1-40) peptide was incubated for five days at 37 °C in (i) phosphate buffer saline (PBS), pH 7.5 [2], and (ii) 10 mM ammoniumacetate buffer, at various pHs, in order to yield oligomers. After the incubation, the aggregates were further characterized by TrisTricin Polyacrylamide Gel Electrophoresis, IMS and MS. The gel electrophoresis analysis ascertained the successful oligomerization of the Aß (1-40) peptide, evidentiating the formation of dimers, trimers, tertramers, and large, high molecular weight aggregates with masses over 200 kDa. Further characterization of the oligomers was made by MALDI-TOF mass spectrometric measurements. The MS spectra confirmed the Aß (1-40) oligomers formation, showing evidence of dimers, trimers and tetramers, in accordance with the gel electrophoreris assay. The ion mobility measurements were of most importance, evidentiating two distinct species with different cross-section areas, and the methionine oxidation at position 36. These new mass spectrometric techniques proved to be of key importance in the elucidation and characterization of a wide range of diseases. This project was supported by the EU-US grant MS-Life (Integrating performance mass spectrometry tools with applications in life science).

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PMo-144
13:30 – 14:40

Higher resolution improves top-down protein ID results on an Orbitrap mass analyzer for large (>40 kDa) proteins

Daisuke Higo[1], David Horn[2], Rosa Viner[2], Seema Sharma[2], Eduard Denisov[3], Alexander Makarov[3]

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Keywords:
Top-down, Protein ID, Orbitrap, ETD, HCD

Novel aspects:
For large intact proteins, the highest possible resolution produces the most confident top-down intact protein identifications.

Abstract:
Introduction
In Fourier transform-based mass spectrometers, there is a trade-off between the acquisition time for a single scan and the resolution of that scan. For bottom-up proteomics experiments, scientists often choose a resolution that is sufficient to resolve the isotopes of the fragments while keeping scan speed as high as possible. Here we study the effect of instrument resolution on the confidence in MS/MS identification of large, intact proteins and it was found that maximizing the resolution rather than maximizing throughput produces the most confident results.
Methods
Bovine serum albumin, enolase, and carbonic anhydrase were obtained from Sigma. The proteins were dissolved in water/acetonitrile at a concentration of 7.5, 10, and 3 pmol/uL, respectively. The proteins were direct infused at 3 uL/min into an Orbitrap Elite system and electron-transfer dissociation (ETD) and high energy C-trap dissociation (HCD) MS/MS spectra were acquired for 1 - 2 minutes at 60,000, 120,000, and 240,000 resolution with 5 microscans per spectrum. A single spectrum was averaged across the full elution profile and Xtract analysis was performed to produce zero-charge masses. The resulting data were searched by ProSightPC and the E-values for the match against the target sequence were calculated.
Abstract
For enolase, three direct infusion experiments were acquired at the three different resolutions mentioned above for ETD of the 44+ charge state of enolase at m/z 1086. For the 60000 resolution experiment, 18 spectra were acquired in the 50 seconds of direct infusion. The subsequent averaged spectrum was matched to the enolase sequence, producing an expectation value of 1.6e-67 matching 49 c ions and 21 matching z ions. For the 120000 resolution experiment, 14 spectra were acquired over 51 seconds and the resulting match produced an expectation value of 1.67e-84 and 58 matching c ions and 33 matching z ions. Finally, the 240000 resolution experiment produced 10 spectra over the 52 seconds of direct infusion and the match produced an expectation value of 5.6e-99 with 55 matching c and 38 matching z ions. Thus, as the instrument resolution was increased, the true fragment matches increased accordingly while the matches to the background signal were reduced, resulting in improved sequence coverage as well as improved confidence.

The higher resolution also enables extension of the range for top down analyses to higher mass proteins. We were able to produce resolved isotope distribution of the 44+ of bovine serum albumin and identify both the HCD and ETD spectra from 1.3 minutes each of direct infusion with highly confident E-values (3.87e-42 for ETD, and 6.27e-19 for HCD) . This hints at the potential for identification of such large proteins on a chromatographic timescale.

We will also present results from LC/MS runs of more complex intact protein mixtures at different resolutions.

PMo-145
11:10 – 12:20

HR/AM Targeted Peptide Quantification on Q Exactive: A Unique Combination of High Selectivity, High Sensitivity and High Throughput

Yi Zhang[2,3], Makoto Takahata[1], Zhiqi Hao[2,3], Markus Kellmann[2,3], Andreas Huhmer[2,3]

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Keywords:
Orbitrap, Peptide Quan, Targeted Approach

Novel aspects:
High sensitivity, high selectivity and high throughput flexible HR/AM targeted quantification on a benchtop Orbitrap mass spectrometer

Abstract:

Introduction

Quantitative proteomics enables the identification of large number of protein candidates, which display biologically interesting dynamics on a global scale in the early discovery phase. Targeted MS approach, in particular selected reaction monitoring (SRM), has become the preferred platform for quantitatively analyzing tens to hundreds of peptide candidates across large number of samples, either for understanding of signaling regulation or for verification and selection of potential clinical biomarkers. In this study, the Q Exactive, a true high resolution and accurate mass (HR/AM) mass spectrometer, was evaluated for targeted protein quantification. The dynamic range and LOD/LOQ of two HR/AM methods, multiplexed targeted selected ion monitoring (msx tSIM) and targeted HCD (tHCD), were investigated.

Methods

Different amounts of 11 heavy isotope labeled yeast peptides from 10 amol to 100 fmol were spiked into either 10ng or 500ng of E coli whole cell digest. The heavy peptides were analyzed with two HR/AM targeted methods, msx tSIM and tHCD, on the Q Exactive™. The targets were quantified at precursor level in msx tSIM method. Up to 4 targets were monitored in a single scan with a resolution of 140,000 (*m/z* 200). The targets were quantified at fragment level in tHCD method. Full HCD spectra were acquired at 12Hz with a resolution of 17,500. The data were automatically processed using Pinpoint with 5 ppm mass tolerance to obtain peak areas, linear dynamic range and LOD/LOQ for each method.

Abstract

For msx tSIM method, the resolution of 140,000 allows baseline separation of two species as close as 30ppm. The high resolution and high mass accuracy (< 5 ppm) ensures confident identification of target ion in complex matrix. The measured isotope distribution is < 5 % from theoretical distribution. Advanced signal processing doubles the resolution for the same transient time, which results in a scan speed of 3 Hz at 140,000 resolution. Two unique features, parallel filling and detection and spectrum multiplexing, further increase total scan speed and throughput. More than eight scans with a resolution of 140,000 were acquired across 12 sec LC peaks even at 10 amol level. A LOD of 10amol and an excellent linear dynamic range of 4 orders were obtained for all heavy peptide targets in a medium complex background of 10ng of E coli whole cell digest. The LOD varied from 50 amol to 1000 amol in a strong complex background of 500ng of E coli whole celldigest. For tHCD method, the high scan speed of 12Hz at a resolution of 17,500 allows acquisition of more than 8 scans across 12 sec LC peaks even at 10 amol level. The high mass accuracy of < 5 ppm and high resolution ensures unmatched confident identification of fragment ions compared to triple quadrupole based SRM method. A LOD of 10amol and an excellent linear dynamic range of 4 orders were obtained for all heavy peptide targets in a medium complex background of 10ng of E coli whole cell digest. The LOD varied from 10amol to 100 amol in a strong complex background of 500ng of E coli whole cell digest.

PMo-146
13:30 – 14:40

Development of an Ion Source for the Analysis of Cuticular Hydrocabons from Living Insects and Steps toward Comprehensive Hydrocarbon Analysis

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Keywords:
ambient, cuticular hydrocarbons, drosophila, UV-LDI

Novel aspects:
An ion source design and sample preparation approaches are presented that could provide a comprehensive spatially-resolved MS analysis of cuticular hydrocarbons from living insects.

Abstract:
Introduction
Insects and other arthropods express species-specific blends of cuticular hydrocarbons (CHCs) on their surface that serve as pheromones and play an important role in behavior / 1 /. Recently, we demonstrated that using ultraviolet laser desorption/ ionization orthogonal time-of-flight mass spectrometry (UV-LDI-o-TOF-MS) enables the spatially-resolved analysis of CHCs from whole Drosophila melanogaster / 2 / and other insects / 3 /. However, one major limitation of this method is that it is typically lethal to the animals. Moreover, so far, alkanes cannot be detected. In a second study, we showed that ion profiles can also be generated from living insects at ambient pressure (AP) solely under the influence of a strong electrical field / 4 /. However, the resulting spectra are extremely complex, presumably due to oxidation reactions.

Methods
In order to address these limitations, we have developed an AP-ion source that provides better control over the ionization conditions (e.g., by applying a protective gas environment and/or adjustable pressure) and the ion transfer into the mass analyzer (QStar-Pulsar, AB-Sciex) . For construction of the source, the particle discriminator approach by Schneider et al. / 5 / was adopted. Capillaries of different diameters and lengths were tested to establish variable flow conditions. Using a coil heater (GC heat) , capillaries can be heated up to 400 ° C. A Nd : YAG-laser (lambda=355nm) can be utilized for UV-LDI. We also tested several strategies for the direct UV-LDI-MS analysis of alkanes and the characterization of double bond positions. Preliminary experiments were performed on intact male and female Drosophila melanogaster.

Preliminary Data
In the poster, details on the design of the ion source and the vacuum interface will be presented. Preliminary experiments demonstrated functionality of the source, including capillary heating and reproduction of our previous MS data obtained by the field-based ion generation (FBIG) method / 4 /. We will show first results on the effect of instrumental parameters (gas pressure, gas type, electrical field strength, capillary design and temperature) on the generation of CHC profiles using FBIG and UV-LDI mass spectrometry at pressures close to 1 bar. To increase the spatial resolution of the "FBIG " method, we will present results from two approaches in which miniaturized extraction capillaries were employed to analyze CHCs from intact insects both with and without activation by a well-focused laser beam.
We will also present data demonstrating that (i) UV-LDI-MS analysis of alkanes is possible after application of silver colloids and (ii) the use of ozonolysis allows partial characterization directly from insect cuticles of the double bond positions of unsaturated hydrocarbons.

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PMo-147
11:10 – 12:20

Oligonucleotide analysis by nanoparticle-assisted laser desorption/ ionization mass spectrometry

Shu TAIRA[1], Daisaku KANEKO[1], Yasuko KONISHI-KAWAMURA-[2], Yuko ICHIYANAGI[3]

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Keywords:
nanoparticle-assisted laser desorption/ionization, oligonucleotide, ionization

Novel aspects:
Reference：S. Taira et al. Analyst vol. 137, 2006-2010 (2012) , (Cover Article)

Abstract:
We analyzed oligonucleotides by nanoparticle-assisted laser desorption/ionization (nano-PALDI) mass spectrometry (MS) . To this end, we prepared several kinds of nanoparticles (Cr-, Fe-, Mn-, Co-based) and optimized the nano-PALDI MS method to analyze the oligonucleotides. Iron oxide nanoparticles with diammonium hydrogen citrate were found to serve as an effective ionization-assisting reagent in MS. The mass spectra showed both $[M\text{-}H]^-$ and $[M+xMe^{2+}\text{-}H]^-$ (Me : transition metal) peaks. The number of metal-adducted ion signals depended on the length of the oligonucleotide. This phenomenon was only observed using bivalent metal core nanoparticles, not with any other valency metal core nanoparticles. Our pilot study demonstrated that ironoxide nanoparticles could easily ionize samples such as chemical drugs and peptides as well as oligonucleotides without the aid of an oligonucleotide-specific chemical matrix (e.g., 3-hydroxypicolinic acid) used in conventional MS methods. These results suggested that iron-based nanoparticles may serve as the assisting material of ionization for genes and other biomolecules.

PMo-148
13:30 – 14:40

Temperature Dependence of Proton Transfer and Conformation Change for Ubiquitin Ions

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Keywords:
temperature, conformation, proton transfer, ubiquitin

Novel aspects:
Temperature dependence of proton transfer for isolated multiply-charged ubiquitin ions with gaseous molecules was investigated. Dramatic change was observed for distribution of product ions and absolute reaction rate.

Abstract:
Temperature dependence ofproton transfer for isolated multiply-charged ubiquitin ions with gaseous molecules of large proton affinity (PA) was investigated with mass spectrometry. A home-made tandem mass spectrometer with electrospray ionization (ESI) was used for measurements. Multiply-charged ubiquitin ions were produced by ESI of a dilute solution of ubiquitin in methanol-water mixture including acetic acid. The ions produced by ESI are admitted into the vacuum chamber through stainless capillary. The charge-selected ions emerging from a quadrupole mass spectrometer are admitted into a collision cell with octapole ion trap. The collision cell is filled with He including gaseous molecules of large PA. We choose 1-propylamine, 1-butylamine, 1-pentylamine, tert-butylamine, or pyridine as target molecules. Temperature dependence of reaction rate and branching fractions for proton transfer from multiply-charged ions to the target molecules was measured, by changing temperature of collision cell. The parent and product ions are mass-analyzed by a time-of-flight mass spectrometer equipped with reflectron.
Proton transfer from ubiquitin ions to the target molecules was occurred by collisions in the cell. Absolute reaction rate for proton transfer was estimated with intensity of ions in the mass spectra. The reaction rate increased with increasing number of the charges (z) . It also increases with increase of PA for the target molecule. By changing temperature of collision cell in region from 280 to 470 K, temperature dependence of reaction rate and branching fractions for proton transfer from ions to target molecules was measured. For ions of z = 6 and 7, dramatic change was observed for distribution of product ions and reaction rate. For z=6, reaction rate increases with decrease of temperature from 450 K to 380 K, whereas it decreases with decrease of temperature from 370 K to 300 K. For z=7, the reaction rate increases rapidly with decrease of temperature from 320 K to 290 K, whereas it decreases rapidly with decrease of temperature from 290 K to 280 K. Change of reaction rates would correlate with conformation of ubiquitin ions, which originates in self-solvation of the proton by hydrophilic residues in polypeptide chains, delocalization of charges with self-solvation, and Coulomb interaction between charges.

PMo-149
11:10 – 12:20

Development of a Dual Pressure Operating Hollow Cathode Glow Discharge Ion Source for Detection of Explosives and Explosive-related Compounds

Md Ahsan Habib, Lee Chuin Chen, Satoshi Ninomiya, Kenzo Hiraoka

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Keywords:
Glow discharge, Explosives, Adduct ions

Novel aspects:
Hollow cathode glow discharge ion source has been proved to be a simple, rapid and robust ionization technique for trace level detection with high selectivity and specificity of multiclass explosives

Abstract:

[Introduction] : Detection of explosives at trace levels is imperative for security purposes because of increasing worldwide terrorist threats at public places. Recently, we have developed a dual pressure operating hollow cathode glow discharge ion source for the analysis of explosives and explosive-related compounds. The ion source was designed in such a way that the plasma can be generated alternatively at high pressure (~1900 Pa) and at low pressure (~140 Pa) regions. The plasma contains a sufficient amount of reactant ions and electrons, and thus the gaseous analyte molecules were efficiently ionized when they passed through the plasma.

[Experimental] : Explosive samples RDX, PETN, TNT, TNB, HMTD, and TATP were purchased as either 0.1 or 1 mg/L acetonitrile solutions. DNT isomers were purchased as solid. Acetonitrile was used for dilution.
Briefly, the glow discharge ion source consists of two metallic tubes of different i.d. (e.g., 2 mm and 5 mm) , and connected with an insulator and an aperture. For sample introduction, a metallic capillary of i.d. ~0.2 mm and 16 mm in length was connected with the tube of i.d. 2 mm. The ion source was coupled with a linear ion trap-MS (Thermo-LTQ) . Mass spectra were collected under automatic gain control with maximum injection time of 10 ms. The targeted species were identified by MS/MS mode. About 1 - 2 µL aliquot from each solution deposited on a glass slide was placed in front of the sample inlet at a distance of 0.5-1.0 cm. The desorbed sample heated between 180-250 °C was introduced into the ion source.

[Results and Discussion] : All the nitro-compounds gave negative product ions under both high pressure plasma (HPP) and low pressure plasma (LPP) conditions, whereas peroxide-based explosives e.g., HMTD and TATP produced only positive ions as either protonated or cluster ions under HPP condition. No product ions were detected for these compounds under LPP condition. Nitroaromatic molecules (M) formed mostly M^- as a product ion under LPP condition at pg level, but, under HPP condition, the compounds formed either M^- or $(M-H)^-$ and also minor ions [e.g., $(M-NO)^-$, $(M-OH)^-$, $(M-H+O)^-$] . The observation of adduct ion of the deprotonated nitroaromatic compounds with O, $(M-H+O)^-$, is our first finding. Moreover, under HPP condition, TNB produced $(M-H)^-$ (100%) , M^- (80%) , $(M-NO)^-$ (~15%) and $(M-H+O)^-$ (~20%) ions, whereas, M^- (100%) ion was produced by TNB when it was introduced with TNT. All DNT isomers produced only M^- (100%) under LPP condition. Under HPP condition, however, 2,4-DNT and 2,6-DNT formed $(M-H)^-$, $(M-NO)^-$ and $(M-H+O)^-$, and 2,3-DNT formed M^- (100 %) and $(M-H)^-$ (60 %) . The dissimilarity in ion formation by the nitroaromatic compounds can be explained by taking the following factors into an account : (i) acidic character of ring and side chain hydrogen, (ii) electron affinity of the nitro-aromatic compounds, and (iii) profusion of reagent ions in HPP region.
For nitro-ester explosives (e.g., PETN, RDX) under HPP condition, PETN produced adduct ion, $(M+NO_3)^-$, whereas RDX formed adduct ions, $(M+NO_2)^-$ and $(M+NO_3)^-$. Formation of only the $(M+NO_3)^-$ adduct ion for PETN suggests the strong bond formation for this cluster ion. Under LPP condition, RDX mostly formed $(M-NO_2-HNO_2)^-$ and $(M-NO_2)^-$, whereas PETN formed $(M-NO_2)^-$.

PMo-150 A Novel Source Design for the Analysis of both Polar and Non-Polar Species

13:30 – 14:40

Steve M Smith[1], Hilary Major[1], Steve Bajic[1], Anthony Bristow[2], Andrew Ray[2]

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Keywords:
novel ionization, non-polars

Novel aspects:
A new API source design capable of enhanced sensitivity analysis of both polar and non-polar species.

Abstract:
Introduction

Since the early commercialisation of API sources, analysts have utilized a combination of ESI, APCI and APPI sources to meet the challenge of ionizing multi-component samples of widely ranging polarities. This approach is hampered by the requirement of different optimal chemistries and the time-consuming need to alternate between source techniques. More recently, multimode source designs have strived to deliver the ultimate goals of broad polarity range applicability, equivalent analyte response and enhanced sensitivity over existing ionization methods. Here, we describe a high-sensitivity multimode API source where ionization is derived from the interaction of a high velocity droplet stream from a grounded probe with a closely coupled high voltage electrode.

Methods

The modified source components were incorporated into a Waters Xevo TQD tandem quadrupole mass spectrometer fitted with an ESI/APCI source housing and electrospray probe. Samples were introduced either by infusion using the system's fluidics or by coupling an ACQUITY UPLC for LC/MS experiments. An electrode voltage of 1 kV was used throughout and other source parameters, such as cone voltage, source temperature and desolvation gas flow rate and temperature, were adjusted according to the flow rate and samples under investigation. This "multi-mode " source has the advantage of ionizing compounds with a wide range of polarities in a single chromatographic run without having to switch ionization modes.
Comparison experiments were carried out using the same instrumentation and standard ESI/APCI and APPI sources.

Preliminary Data
This novel source has been found to be more sensitive than electrospray (typically 3) for the majority of polar species investigated such as peptides, pesticides and pharmaceuticals.
The source has also been used to analyse a range of non-polar compounds more commonly analyzed by APCI and APPI and has shown an improvement in sensitivity over these techniques. The results obtained for vitamins and poly-aromatic hydrocarbons (PAHs) show the principal ions generated are $M^{+\cdot}$ radical cations and $[M-H]^+$ ions by hydride abstraction while steroids generate $[M+H]^+$ ions.
Data will be presented demonstrating the range of compounds which can be analysed by this source and the gain in sensitivity achieved.

PMo-151 Sheath- flow PESI for Dry Samples

11:10 – 12:20

Md Obaidur Rahman, Yasuo Shida, Mridul Kanti Mandal, Satoshi Ninomiya, Lee Chuin Chen, Kenzo Hiraoka

University of Yamanashi,Kofu,Japan

Keywords:
Electrospray, PESI, Sheath-Flow PESI

Novel aspects:
Probe electrospray ionization (PESI) associated with sheath-liquid flow is newly developed. This technique can be applicable to any samples regardless of their wetness.

Abstract:
Introduction :
Probe electrospray ionization (PESI) uses a solid needle or a wire as a sampling probe and an ESI emitter instead of a capillary. PESI can be applicable to various biological samples without any special sample pretreatment. It was also found that PESI was superior to nanoESI for samples with high concentration of salts, urea, and detergents. One drawback of PESI is that it is not applicable to dry samples. In our laboratory, PESI coupled with a coaxial capillary, namely, sheath-flow PESI, was newly developed. By supplying solvent through the capillary, this technique was found to be applicable to any samples regardless of their wetness.

Experimental :
An acupuncture needle (0.12 mm o.d.) was inserted into the fine glass or plastic capillary with i.d. of 0.3 mm. The acupuncture needle was protruded from the tip of the capillary by 0.2-0.3 mm in length. The solvent such as methanol was flowed through the capillary with flow rate of 1 microL/min (one micro litre/min) or less. When the PESI probe slightly touches the sample surface, the solvent flowing out of the capillary makes the sample wet with the solvent and sample components were extracted to the solvent liquid. After the sample extraction, the PESI needle was moved to the highest position. By the application of high voltage of about 2.5-3 kV to the PESI probe, the extracted samples were electrosprayed. Mass spectra were measured by a time-of-flight mass spectrometer (JEOL, AccuTOF) .

Results and Discussion
Because the solvent was continuously supplied to the tip of the acupuncture needle, this method can be applicable to dry and also wet samples. Some examples are given below.
[Tablets] : Sheath-flow PESI was applied to several tablets, such as loratadine, atenolol, etizolam, triazolam, etc. The protonated molecule ions were observed as major ions for all the tablets examined.
[Human finger] : By touching the volunteer's finger by the probe, intense signals originating from lipids and sometimes detergents were detected. Dry saliva and urine also gave strong ion signals.
[Bank notes] : A US dollar bill was scanned/analyzed from the central to the terminal positions. In the central part of the bill, little signals were observed. However, the signal of protonated cocaine was clearly observed at the edge of the bill.
[Plants] : Characteristic mass spectra could be obtained from plants such as vegetables, fruits etc. When a greenish potato was examined, a strong signal of toxic solanine was detected on the surface, while no such signal was detected in the central part of the sample. The insecticides and fertilizers in plants could also be detected by this technique.
[Illicit drugs] : After the methanol droplet containing 100 pg of methamphetamine or morphine was deposited on the finger, the dried area (3 mm in diameter) was examined. Strong signals for both compounds could be detected.

The sheath-flow PESI can be applicable to any samples regardless of wetness. By using meter-long flexible capillary, it may be used as a very fine endoscope. After the sampling with least invasion, the PESI mass spectra can be obtained by sheath-flow PESI.

PMo-152 An Electron Impact Ion Source for a Miniature Mass spectrometer

13:30 – 14:40

yide zhao, meiru guo, liang wang, yuhua xiao, taiguo li

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Keywords:
Electron Impact；SIMION；optimal design

Novel aspects:
Thought optimal design by SIMION, The performance of the EI ion source combined with miniature magnetron sector mass spectrometer was tested.

Abstract:
Miniature mass spectrometer, one of the most powerful and versatile methods of real time and in suit chemical analysis, is widely utilized in harsh environment, such as space exploration, active volcanoes, underwater environment, and the battlefield. A miniature magnetic sector mass spectrometer has been developed that consists of an electron impact (EI) ion source, a magnetic mass analyzer, two continuous dynode multiplier detectors, and associated electronics circuits.

In this paper, the design, simulation and construction of the EI ion source is described, which consists of a small chamber attached to a rigid mounting plate through which an electron beam is produced by thermal emission from tungsten filament. A pair of collimation magnets mounted to two walls of the chamber which creates the field for the alignment of the electron beam. Ions produced in the source are drawn out by a high voltage and collimated into a beam by a series of electrostatic lens system which consists of repeller, exit slit of ionization chamber, extraction electrodes, focusing electrodes, main slit and alpha slit.

Charged particle electrodynamic modeling (SIMION 8.0) was employed to simulate the ion source electrostatic lens system. The beam emittance and beam width as a function of the lens system voltage ratio for single charged ion trajectories have been investigated. Influence of the atomic masses of the different elements has been studied on both the beam emittance and beam width.

Simulation results were used to verify and optimize the performance of the ion source before and during its fabrication. The performance of the EI ion source combined with miniature magnetron sector mass spectrometer was tested.

PMo-153
11:10 – 12:20

Development of High Pressure Ion Sources with Operating Pressure Higher Than Atmospheric Pressure

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Keywords:
High pressure ion source, electrospray

Novel aspects:
Development of high pressure ion source with operating pressure high than one atmospheric pressure using ionization techniques such as ESI, CI and FD.

Abstract:
Early attempts to couple ESI ion source with mass spectrometry were conducted by placing the ion source in high vacuum. Due to insufficient bath gas and desolvation, vacuum ESI suffered from poor sensitivity and could only handle limited types of solvents. John Fenn et al. revolutionized the ESI method by bringing the ion source to the atmospheric pressure ambient, and the bath gas provided sufficient thermal energy to the charged droplets for the vaporization of solvent without freezing them. Nowadays, standard ESI ion source is operated under an atmospheric pressure and the mass spectrometer, which is designed to work with ESI and other atmospheric pressure ionization (API) source, is referred as API mass spectrometer. To date, nearly all ion sources (e.g. MALDI, ESI, APCI, EI, CI, FI/FD, etc.) are operated either under high vacuum or one atmospheric pressure.

Super-atmospheric pressure electrospray ionization with operating pressure > 5 atm has recently been demonstrated in our lab [1,2] . By raising the operating air pressure of the ion source, we have verified that the threshold for the corona and arc discharge increases but the threshold for the onset of electrospray remained unchanged. This is an interesting advantage because it allows a stable electrospray ionization to be performed without the occurrence of disturbing corona and arc discharge even for pure water solution which is of high surface tension.

In this presentation, we will report some of our recent progress on the development of high pressure ion source with ionization techniques other than electrospray. Some of our efforts have been put on the ionization of gaseous compounds because the number of gas phase interaction is increased with the increase of pressure. Also, by sufficiently quenching the electrical discharge by increasing the ion source pressure, some of our preliminary results have showed that it is even possible to perform stable non-vacuum field desorption (FD) which is until now has to be performed under ultra high vacuum.

Reference
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PMo-154
13:30 – 14:40

Conformation Change for Insulin and Insulin Chain B Ions; Temperature Dependence of Proton Transfer

Ayako Sudo, Keishi Machida, Kanako Yokoyama, Minami Kawashima, Takashige Mori, Yuto Ohshima, Kazuki Yamashita, Shinji Nonose

Yokohama City University

Keywords:
temperature dependence, proton transfer, insulin

Novel aspects:
Temperature dependence of proton transfer for insulin and insulin chain B ions with gaseous molecules was investigated. Dramatic change was observed for distribution of product ions and absolute reaction rate.

Abstract:
Temperature dependence ofproton transfer for isolated multiply-charged insulin and insulin chain B ions with gaseous molecules of large proton affinity (PA) was investigated with mass spectrometry. A home-made tandem mass spectrometer with electrospray ionization (ESI) was used for measurements. Multiply-charged insulin and insulin chain B ions were produced by ESI of a dilute solution in methanol-water mixture including acetic acid. The charge-selected ions emerging from a home-made quadrupole mass spectrometer are admitted into a collision cell with octapole ion trap. The collision cell is filled with He including gaseous molecules of large PA. We choose 1-propylamine, 1-butylamine, 1-pentylamine, tert-butylamine, or pyridine as target molecules. Temperature dependence of reaction rate and branching fractions for proton transfer from multiply-charged ions to the target molecules was measured, by changing temperature of collision cell. The parent and product ions are mass-analyzed by a time-of-flight mass spectrometer equipped with reflectron. Reduction of disulfide bonds in insulin to produce insulin chain B ion was carried out with 1,4-dithiothreitol (DTT) . A small amount of DTT was added to insulin aqueous solution (-0.1 mM) in distilled water, such that the final DTT concentration was approximately 1 mM. After a period of over 20 minutes at 350 K, mass spectra of insulin chain B ions were obtained.

Proton transfer from insulin and insulin chain B ions to the target molecules was occurred by collisions in the cell. Absolute reaction rate for proton transfer was estimated with intensity of ions in the mass spectra. The reaction rate increased with increasing number of the charges (z) . It also increases with increase of PA for the target molecule. By changing temperature of collision cell in region from 290 to 470 K, temperature dependence of reaction rate and branching fractions for proton transfer from ions to target molecules was measured. For $z = 4$ of insulin ion reacted with pyridine, dramatic change was observed for distribution of product ions and reaction rate. The reaction rate decreases with decrease of temperature from 450 K to 330 K, and it increases with decrease of temperature from 330 K to 290 K. For $z = 3$ of insulin chain B ion reacted with pyridine, reaction rate increases rapidly with decrease of temperature from 350 K to 290 K, whereas the reaction rate with 1-butylamine, 1-pentylamine or tert-butylamine decreases gradually with decrease of temperature from 450 K to 280 K. Proton transfer would proceed by two-step mechanism ; complex formation of ions with target molecules, and release of protonated target molecular ion. These reaction rates would correlate with conformation change of insulin and insulin chain B ions with change of temperature. The conformation change would originate in self-solvation of the proton by hydrophilic residues in polypeptide chains.

PMo-155
11:10 – 12:20

Development of high sensitive low vacuum dielectric barrier discharge ionization method

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Keywords:
Dielectric_barrier_discharge

Novel aspects:
Development of high sensitive low vacuum dielectric barrier discharge ionization method

Abstract:

Introduction

Recently, ionization methods using dielectric barrier discharge (DBD) have become of great interest [1, 2] . In this study, we developed a novel high sensitive DBD ionization method, which is called a low vacuum DBD ionization method. In this configuration, samples directly pass through the inside of the DBD plasma where charge density is high. Because the ion source is placed in a vacuum, the conductance between the ion source and the mass spectrometer is large, which leads to high introduction efficiency. High ionization efficiency and high introduction efficiency result in high sensitivity.

Method

The low vacuum DBD ion source consists of a glass tube, a tube-shaped discharge electrode, and a ring electrode. The grounded discharge electrode was inserted into the glass tube, and the ring electrode was attached around the glass tube. The AC voltage of 2 kV was applied to the ring electrode. The DBD plasma was generated between the end of the discharge electrode and the ring electrode.

The instrument consists of the low vacuum DBD ion source and an ion trap. Vaporized samples were discontinuously introduced from ambient air into the ion source and passed through the inside of the plasma directly where gas molecules were converted to ions. The ions produced were introduced into the ion trap and were mass analyzed.

Results

The mass spectra of methyl salicylate were measured by using a low vacuum DBD ion source. The ambient air introduced with the samples gas was used as a discharge gas. Water cluster ions ((H2O) nH+) n = 1, 2, 3) and [M+H] + ions of methyl salicylate were observed as dominant ions. This result shows that the low vacuum DBD ion source is a soft ionization method.

To optimize the pressure of the region where ionization occurred, the intensity of the [M+H] + ions of methyl salicylate was measured as a function of ion source pressure. The intensity of the [M+H] + ions became maximum when the pressure of the region where ionization occurred ranged from 1000 to 3000 Pa.

The intensity of the [M+H] + ions of methyl salicylate observed by using low vacuum DBD ion sources was 30 times higher than that observed by using conventional atmospheric pressure chemical ionization (APCI) ion source when we set the flow rate to be the same for both configurations.

At the conference, we will report experimental results of other samples by using a low vacuum DBD ion source.

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PMo-156 High ion yields of carbohydrates from frozen solution by UV-MALDI

13:30 – 14:40

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Academia Sinica, Taipei, Taiwan

Keywords:
MALDI, carbohydrate, frozen solution,

Novel aspects:
High ion yiels and low fragmentation from frozen solution by UV-MALDI

Abstract:
Aqueous acetonitrile solution containing oligosaccharide (maltopentaose) and matrix (2, 5-dihyrroxybenzoic acid) was frozen at 100 K for ultraviolet matrix-assisted laser desorption ionization (UV-MALDI) mass analysis. Compared to the conventional UV-MALDI of dried analyte and matrix mixture (after evaporation of solvent) , frozen solution generates more oligosaccharide ions and less fragments from post-source decay. It is long-lasting and the analyte distribution is more homogeneous. The ion generation efficiency is 20-30 times larger than conventional dried mixture. The percentages of fragmentation due to post-source decay for frozen samples are almost zero (< 2 %) , compared to 17% and 40% for dried samples at low and high laser fluences, respectively. The applications to polysaccharides also demonstrate that the ion intensity from frozen solution is 20 times larger than conventional method.

PMo-157
11:10 – 12:20

ATMOSPHERIC PRESSURE PHOTOIONIZATION-MASS SPECTROMETRY OF ESTROGENIC COMPOUNDS

Oscar Nunez[1], Paolo Lucci[2], Encarnacion Moyano[1], Maria Teresa Galceran[1]

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Keywords:
APPI, UHPLC, Mass Spectrometry, Estrogenic Compounds,

Novel aspects:
Study of the effect of different dopants in the APPI ionization of estrogenic compounds

Abstract:
Among endocrine-disrupting chemicals (EDCs) , natural and synthetic estrogens are considered the most potent estrogenic compounds. EDCs are widely distributed over the aquatic environment and due to their ecotoxic effects natural and synthetic estrogens are of special relevance even at very low concentrations. Estrogenic compounds are usually excreted into the aquatic environment through human and animal urine and the use of estrogens in medicine or in veterinary have caused their presence in aquatic ecosystems [1] . Liquid chromatography coupled with tandem mass spectrometry using both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) is the technique of choice for the LC-MS analysis of these compounds [2,3] . However, estrogens are non polar compounds and are usually hard to ionize using classical LC-MS sources mainly ESI. Atmospheric pressure photoionization (APPI) which can ionize both polar and non polar molecules with high sensitivity can be considered an alternative and has began to be used for the analysis of these compounds [4] . An additional advantage of using APPI is that ionization can be enhanced adding dopant substances and in some cases the choice of an adequate dopant is as much important as the choice of the source of ionization itself [5] .

The aim of this study was to compare the behavior of three ionization sources (ESI, APCI and APPI) on analyzing eight estrogenic compounds (dienestrol, diethylstilbestrol, estrone, 17β-estradiol, 17α-estradiol, mestranol, 17α-ethinylestradiol and estriol) . LC-MS was carried out using an Accela liquid chromatograph system coupled to a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (ThermoFisher Scientific) . To evaluate APPI performance six dopant solvents (toluene, acetone, ethylacetate, anisole, clorobenzene, tetrahydrofuran) as well as some dopant mixtures (toluene-anisole and tolune-chlorobenzene) were used and their sensitivity and their capability to ionize estrogens was compared. Negative ESI mode only provided the deprotonated molecules as base peak and low responses while in positive APCI the dehydrated fragment ion was found for the majority of the estrogenic compounds and better signals were obtained. As regards APPI in general most of the dopants provided the positive radical ions as the most abundant peaks. However, when using anisole or the mixture toluene-anisole as dopants the protonated ion $[M+H]^+$ resulted favored against the positive radical ions. For all the estrogenic compounds the highest signal responses were found with chlorobenzene and the mixture toluene-chlorobenzene except for dienestrol that gave higher signals using anisole, although signal-to-noise ratio worsened due to dopant solvent ionization. The detectability of the selected estrogens by APPI using chlorobenzene was found to be more effective than that of APCI and ESI, and was then selected for the analysis of estrogenic compounds.

A fast and high-efficient chromatographic separation of all estrogenic compounds was achieved in less than 4 min with an Ascentis Express Phenyl-Hexyl HPLC column (150 mm x 2.1 mm i.d., 2.7 µm) and gradient elution using water and acetonitrile as mobile phases. Good method performance was observed in terms of LODs (ppt level) , linearity and run-to-run precision (RSD < 4 %) . The method was applied to the analysis of wastewater samples from a Hospital after off-line SPE using HLB cartridges, and estrone and estriol were detected and quantified at low ppb levels.

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PMo-158 Application of Electrospray Droplet Impact / SIMS to synthetic polymers

13:30 – 14:40

Rio Takaishi, Yuji Sakai, Satoshi Ninomiya, Kenzo Hiraoka

Clean Energy Research Center, University of Yamanashi, Yamanashi, Japan

Keywords:
Electrospray Droplet Impact ; SIMS ; Cluster ion ; synthetic polymers

Novel aspects:
EDI is capable of atomic- and molecular level etching without leavingthe damage after irradiation. It would be a valuable technique for thenext-generation surface science.

Abstract:
The electrospray droplet impact secondary ion mass spectrometry (EDI/SIMS) has been developed as cluster SIMS [1]. The typical primary droplets are roughly represented as $[(H_2O)_{90,000} + 100H]^{100+}$ (mass of 1.6×10^6 u). The kinetic energies of charged water droplets are 10^6 eV with the velocity of ~12 km/s. EDI/SIMS has the atomic/molecular level etching ability with high ionization efficiencies. Furthermore, degradation products do not accumulate on sample surfaces (i.e. surface-cleaning effect) [1]. EDI/SIMS has been applied to many inorganic and organic samples. For synthetic polymers, EDI mass spectra were found to reflect the backbone structures for the polymers [2] [3]. In addition, no selective etching [2] [3] was observed for all samples examined, such as PS, PI, PET, PMMA, etc.

Synthetic polymers are the target for the active investigation in semiconductor field, for example, package of semiconductor, flexible printed circuit, flexible display etc. Analysis of thin-film synthetic polymer used in the semiconductor field is of significant importance. In this paper, the application of EDI to thin and bulk samples of various synthetic polymers will also be given. The comparison of etching rates for organic and inorganic materials will also be given.

Reference

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PMo-159
11:10 – 12:20

Development of high pressure (>1 atm) probe electrospray ionization mass spectrometry

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Keywords:
PESI, HP-PESI, gas breakdown,

Novel aspects:
HP-PESI can suppress the occurrence of the gas breakdown. It is readily applicable to aqueous sample solutions in both positive and negative mode of operations.

Abstract:
Probe electrospray ionization (PESI) has been developed in our laboratory [1-4]. It can be applicable to wet real-world samples without any sample pretreatment. Because it uses a fine metal needle, the gas breakdown is apt to take place for aqueous sample solutions, especially in the negative-mode of operation. In order to circumvent this problem, a high-pressure PESI (HP-PESI) was developed.
The PESI ion source chamber was pressurized up to 7 bars with dry air supplied from an air compressor. To couple the high pressure ion source directly to the mass spectrometer, the default ion transfer tube of the ion trap mass spectrometer (Thermo, Velos) was replaced with a custom made one with 0.25mm in id and 1/16 " in od. The desolvation chamber was made of aluminum alloy that has two view ports made of polycarbonate for the optical microscopic observation.
HP-PESI was found to have several advantages. First it can provide denser heat bath gas for the desolvation of the charged droplets. Second, the gaseous breakdown was almost totally suppressed in both positive- and negative-mode of operations. These features are particularly useful for the analysis of peptides and proteins in aqueous solutions.
A comparative study of PESI and HP-PESI was performed. HP-PESI was found to be superior to PESI for the aqueous sample solutions in the negative-mode of operation because negative-mode PESI measurements were sometimes disturbed by the occurrence of corona discharge at the tip of the needle. HP-PESI would be an attractive tool particularly for the analysis of native proteins because it can handle the aqueous solutions that contain no organic solvents.

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PMo-160

13:30 – 14:40

Development of an Atmospheric Pressure Laser Ionization Technique using a Novel 6 um-band Mid-Infrared Tunable Laser and Liquid Water Matrix

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Keywords:

Mid-infrared tunable laser, Atmospheric pressure ionization, Water, Matrix-free, O-H bending vibration

Novel aspects:

We ionized peptide in aqueous solution under atmospheric pressure by irradiation of mid-infrared laser with a wavelength around 6 um and peak intensity was strongest at the wavelength 5.95 um.

Abstract:

Purpose: The purpose of this study is to develop a new laser ionization method using absorption corresponding to O-H bending vibration of water and to evaluate the wavelength dependence of the ion signal intensity. Ionization of peptides in aqueous solution by using a mid-infrared laser with the wavelength of 3 μm corresponding to the O-H stretching vibration under the atmospheric pressure has been demonstrated[1) 2)]. On the other hand, the 6 μm-band mid-infrared has advantages compared to the 3 μm-band as follows :

Various molecular vibration modes such as O-H bending vibration, C=O and C=N stretching vibration have characteristic absorption in the 6 μm-band. Thus, not only water but also various solvents and substances can be used as matrix. In addition, the change in the absorption peak caused by the temperature rise for the O-H stretching vibration at the wavelength of 2.94 μm.

However, the atmospheric pressure ionization using 6 μm-band mid-infrared laser has never been reported bacause it has been difficult to obtain a high power tunable laser in the 6 μm-band. We have investigated using a 6 μm-band mid-infrared tunable laser which has recently been developed.

Materials and Methods: A mid-infraraed tunable pulesed laser using difference-frequency generation (DFG) was used. The mid-infrared DFG laser has a tunable wavelength range of 5.5-10.0 μm, a repetation rate of 10 Hz, and a pulse width of 5 ns. The tunable range of this laser includes the absorption peak of water corresponding to the O-H bending vabration at the wavelengh of 6.07 μm.

In this research, atmospheric pressure laser ionization ion source was constructed and assembled on the commercial ion trap mass spectrometer (LCQ Classic, Thermo Finnigan) . A heated capillary of the ion trap mass spectrometer was extended with a self-produced extension capillary, and the liquid sample on the stainless plate in front of the extension capillary was irradiated with DFG laser. Angiotensin (1045.54 Da, SIGMA-ALDRICH) was dissolved in 0.1% solution of trifluoroacetic acid (TFA) in water and adjusted to a concentration of 10 pmol/μL. A drop of this sample solution (1 μL) was dropped on the sample plate and laser irradiation and acquiring mass spectrum was started within 1 minute after dropped. Laser energy was 250 μJ/pulse, and injection time of ion trap was 300 ms and measured 30 mass spectra were averaged.

Results and Discussion: Mass spectra of protonated angiotensin II ($[M+H]^+$) were observed around the wavelength 6.07 μm corresponding to O-H bending vibration mode. Ionization was not observed on the wavelength of 5.75 μm or less. Wavelength dependence of the peak intensity of angiotensin II was similar to the IR absorption spectrum of liquid water. This result indicated that liquid water functioned as matrix. However, peak intensity at the wavelength of 5.95 μm was stronger than at the wavelength around 6.07 μm. As a reason for this experimental result, it is supposed that the pulse energy of 250 μJ is too high at the wavelength around 6.08 μm. In this method, aromatic matrix such as α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) were not mixed into sample solution. Liquid water as solvent was utilized as also matrix. Therefore, this technique showed promise for the application as an interface connecting liquid chromatography (LC) and mass spectrometer. In the future work, laser irradiation condition will be optimized and online connection between LC and mass spectrometer by this ionization technique will be investigated.

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PMo-161
11:10 – 12:20

Influence of source parameters on the detection and the fragmentation of aroma compounds using PTR-SRI-ToF MS

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Keywords:
PTR-SRI-MS ; Flavouring Process ; Flavour Release ; Switchable Reagent Ion ; Real-Time Aroma Perception

Novel aspects:
Using Proton Transfer Reaction-Switchable Reagent Ion-Mass Spectrometry to measure headspace of aroma (in-vitro) and flavour release in the expired air (in-vivo) to understand aroma perception and improve flavouring processes

Abstract:
Acceptability or rejection of foods by consumers highly depends on their aroma quality. Food aroma is a complex mixture of several volatile compounds in a wide range of concentration and of chemical structure. During consumption, aroma release from foods is a complex sequential process involving interactions of flavour molecules with the matrix, the saliva and the chewing activity. The gas phase is transferred to the nasal cavity where it has to reach the sensory receptors to be perceived. The gaseous atmospheres in the air above foodstuff (*in-vitro* conditions) and created during food consumption in the oral cavity (*in-vivo* conditions) may be significantly different. Studying these two aspects is essential to investigate flavour release before and during eating and ultimately to understand sensory perception. Proton Transfer Reaction mass spectrometry (PTR-MS) is a very efficient technique to continuously monitor volatile organic compounds. It can be advantageously used to analyse the headspace of an aroma solution (*in-vitro* conditions) but also air in the nasal cavity during eating (nosespace) , taking into account the dynamics of aroma release induced *in-vivo*.
Proton Transfer Reaction mass spectrometry (PTR-MS) is a specific chemical ionization using H_3O^+ as precursor ion. H_2O is firstly ionized in the ion source to create H_3O^+ by electron impact. Then, reagent ions react with neutral molecules into a drift tube mainly leading to the production of $[M+H]^+$ protonated molecules. The reaction of proton transfer is only possible for analyte molecules with a proton affinity (PA) superior to the one of water (PA_{water} = 165.1 kcal.mol^{-1} ; IP_{water} = 12.62 eV) . The PA consideration limits ionization opportunity. Thus a Switchable Reagent Ions (SRI) system on the PTR-MS source (Ionicon Analytik, Innsbruck) enables to switch to other precursor ions *i.e.* O_2^+or NO^+. Hence, charge transfer ionization is possible for molecules with a lower ionization potential than the one of O_2 (PA_{O2} = 100.6 kcal.mol^{-1} ; IP_{O2} = 12.06 eV) . The use of NO^+ mainly leads to dehydrogenated cations $[M-H]^+$ (PA_{NO} = 125.7 kcal.mol^{-1} ; IP_{NO} = 9.26 eV) . In addition to the SRI system, detection and fragmentation of analytes can be highly modified by varying drift tube parameters and more particularly the E/N ratio (E : electric field strength in the drift tube ; N : buffer gas number density in the drift tube) . The E/N ratio is interlocked with the physico-chemical nature of the analytes and then to the mass spectra pattern through fragmentation and needs to be adapted to the applications.
The aim of this work was to analyze the impact of the precursor ions produced in the SRI source combined with several E/N ratios to optimize the analyses of aroma compounds. Analyses were conducted on the headspace of twelve different aroma compounds belonging to various chemical classes, chosen for their frequent use in flavouring. Comparison of the signal response was followed according to the reagent ion selected and 15 E/N ratios, focusing on the intensity, the sensitivity, the resolution and the fragmentation pattern of the analytes under every experimental condition. The results highlighted that both reagent ion and optimal E/N parameter were closely linked to the chemical structure of the ionized molecules and that experimental parameters have to be adapted to the applications, *i.e.* more or less fragmentation needed. When optimal parameters are determined, *in-vivo* experiments can be realized.
These first *in-vitro* and *in-vivo* results show the massive potential of the PTR-SRI-MS technique to study flavour release in order to understand aroma perception and improve flavouring processes in food industry.

PMo-162
13:30 – 14:40

Expansion of an ionization technique in nonpolar solvents with cation tetrakis(3,5-bis(trifluoromethyl)-phenyl)borate

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Keywords:
nonpolar solvent, ionization method, cationization reagent, electrolyte, nebulizer

Novel aspects:
An ionization method, developed in our laboratory, in nonpolar solvents with using cation tetrakis (3,5-bis (trifluoromethyl) -phenyl) borate (cation-TFPB) was improved by using a biological characteristic and interfacial chemical reactions.

Abstract:
Generally selection of ionization method changes by molecular species or experimental condition. The most efficient technique for one molecule is not useful for other molecules in many cases. In weakly polar molecules, it is difficult to observe a large part of these molecules by conventional methods. We have studied a novel method to enable to ionize a lot of weakly polar molecules softly. This method has two important features using nonpolar solvent and cationization reagent (electrolyte) . Recently, it was found that electrolytes containing tetrakis (3,5-bis (trifluoromethyl) phenyl) borate anion ($TFPB^-$) were useful as cationization reagents to lipophilic analytes in nonpolar solvents. The TFPB anion possesses two characteristic properties, a large lipophilicity and a small surfacecharge density, that result from the anion's size ; this makes the anion readily soluble in nonpolar solvents with a small donor number (DN) , such as dichloromethane and toluene. In most cases, cation or anion hardly exists independently in nonpolar solvent. Above cationzation reagents (Li^+,Na^+ and Ag^+ $TFPB^-$) , however, can slightly dissociate ; unstable dissociated cations easily can react with analyte molecules and form ion pair with $TFPB^-$. This reactivity depends on chemistry between an analyte molecule and a cation ; the improvement of reaction efficiencies closely connects with increase of measurement sensitivity. In this research, we discuss some methods for the improvement of reaction efficiency concerning cation-analyte reaction in nonpolar solvents.
It is expected to enable ionization of only molecules containing a certain functional group by the suitable selection of cation species. To achieve this selective ionization, we paid attention to a molecular selectivity in biological systems. The selectivity in vivo is precise, and can be available for selective ionization. First, we focused on 8,13-Bis (ethyl) -3,7,12,17-tetramethyl-21H,23H-prphine-2,18-dipropionic acid iron (III) chloride Mesohemin (Fe (III) Mesoporphyrin IX chloride) , to synthesize electrolyte containing a $TFPB^-$. This molecule resembles heme closely and may combine to oxygen atom selectively. In fact, our experimental results showed that Fe (III) Mesoporphyrin -TFPB had strong Lewis acidity for molecules with hydroxyl functional group (-OH) .
A liquid-liquid interface and a liquid surface are different from the bulk due to unbalanced forces exerted on particles in this region. We built a new sprayer with triple tube to use these conditions. A conventional sprayer is double tube and can flow a sample solution and nitrogen (N_2) . In a new triple tube, other one solution or air can be flowed ; a liquid-liquid or a bubble-liquid interface can be formed in the inside of the sprayer. With our ionization method using the conventional sprayer, cationized multimeres are produced in many cases. With the triple tube, a cationized monomer is mainly observed. These result indicate that the ionization process of analyte molecules in triple tube is different from the case of using the conventional sprayer.
Experimental
Cationization reagents were synthesized from sodium tetrakis (3,5-bis (trifluoromethyl) phenyl) borate (NaTFPB, purchased from Dojindo) and salts containing objective cations by a solvent extraction method. Sample solutions were injected by syringe pump and nebulized by a sprayer (in-house built) to form fine droplets. The sprayer was positioned parallel to the axis of a sampling orifice at a distance of about 1 mm from the orifice.

PMo-163
11:10 – 12:20

Gas chromatographic separation and accurate mass determination by low temperature plasma ionization API-QTOF-MS

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National Research Centre for the Working Environment, Denmark

Keywords:
Low temperature plasma ; Gas chromatography ; GC interface ; Adduct ion formation ;

Novel aspects:
A sensitive and versatile interface between a GC and an API-QTOF-MS based on low temperature plasma ionization facilitating mass measurements with accuracies of 5 mDa.

Abstract:

Introduction

Low temperature plasma (LTP) is a recent ambient ionization technique where cold plasma (~30 ° C) is utilized for direct mass spectrometric analysis of liquid and solid samples, and compounds in the gas phase. The plasma is generated inside a small glass tube by an alternating high voltage applied to an outer ring electrode, a grounded center electrode and a low flow (< 0.3 l/min) of discharge gas (He, Ar, N_2 or air) . LTP is based on dielectric barrier discharges that are produced when a high voltage applied to electrodes with dielectric insulators causes a series of micro-discharges due to electrical breakdown of discharge gas between the electrodes. This series of micro-discharges induces a cascade of reactions that form a non-equilibrium low temperature plasma rich in metastable neutrals as well as ionized species. When He is used as discharge gas, He_2^+ is the dominant positive ion in the discharge region inside the glass tube. As the plasma enters the open atmosphere, N_2^+ and subsequently water clusters $(H_2O)_nH^+$ are formed by charge transfer reactions. Thus, LTP ionization in the positive mode usually yields protonated molecules, however adduct ions such as $[M+OH]^+$, $[M+NO]^+$ and $[M+NO_2]^+$ may also be formed to some extents.

Since it's launching in 2008 LTP has been used for a wide range of applications, e.g. coating products, explosives, pharmaceuticals and olive oil. Compounds of interest are liberated from surfaces by thermal processes, thus LTP works best for compounds with a vapour pressure. Compounds that elutes from a gas chromatograph (GC) should therefore be ideal for ionization by LTP. In order to exploit the facilities for accurate mass measurements offered by an atmospheric pressure ionization quadrupole time-of-flight mass spectrometer in combination with a GC, LTP was used as an interfacing ionization technique.

Experimental

An LTP probe was mounted in a modified APCI source and carefully aligned with a GC-column in front of the inlet orifice. A heated transfer line was utilized in order to keep the column temperature at 225 °C from the GC to the ion source. Sample volumes of 1 ml were introduced via split-less injection onto a 30 m Agilent VF-5 column with an inner diameter of 0.25 mm. The mass spectrometer, a Bruker micrOTOF-Q, was operated in the positive mode and spectra were recorded at rate of 1 Hz. A mass accuracy of 5 mDa was obtained by lock mass calibration using phthalic anhydride (*m/z* 149.0223) as calibrant.

Preliminary results

Aromatic compounds, terpenes and terpenols in a test solution (dichloromethane) were successfully baseline separated. The GC peaks had widths of 7-14 s and the limit of detection was about 0.5 ng for most compounds. Protonated molecules $[M+H]^+$ was observed as the most abundant ion for all compounds. However, radical cations $M^{+\cdot}$ and adduct ions such as $[M+OH]^+$, $[M+O_2H]^+$, $[M+NO]^+$ and $[M+NO_2]^+$ were also observed as abundant ions for several compounds. The number and type of observed ions differed with the analyte. For instance, a-pinene showed $[M+H]^+$, $[M+OH]^+$, $[M+NO]^+$ and $[M+NO_2]^+$, while xylene showed $M^{+\cdot}$, $[M+H]^+$, $[M+OH]^+$ and $[M+O_2H]^+$. In general, the compound groups yielded different adduct ion patterns. These differences in adduct ion formation might be useful for the identification of functional groups in analyte molecules.

PMo-164
13:30 – 14:40

Fast, High Throughput LC-MS/MS Method for Immunosuppressants Analysis Using Dual Probe Electrospray

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IONICS Mass Spectrometry, Toronto, Canada

Keywords:
Immunosuppressants ; dual source ; mass spectrometer

Novel aspects:
Demonstration of a highly sensitive dual electrospray ion source method that optimizes throughput for clinical settings

Abstract:

Objective

Immunosuppressant panels are now commonly used by clinical mass spectrometrists for patient management, for example to ensure succesful organ transplants. A fast, sensitive, and accurate LC-MS/MS quantitation method is presented using a novel, highly sensitive dual probe electrospray ion source for analysis of Tacrolimus, Sirolimus, Everolimus and Cyclosporin A using Cyclosporin D and Ascomycin as internal standards.

Methods

Whole blood spiked samples were cleaned up by mixing one volume of serum with two volumes 0.1 M $ZnSO_4$ precipitation solution containing the internal standards (Cyclosporin D and Ascomycin). Three level QCs were purchased from UTAK (Valencia, CA). The mixture was vortexed for one minute followed by centrifugation for 15 min. The supernatant was transferred to a clean vial for quantitation by two-dimension LC-MS/MS. 20 µL of supernatant were loaded on a Poros R1/20 pretreatment column (30x2.1mm) for on-line washing with water for a half minute at a liquid flow rate of 3 mL/min, then eluted by an Imtakt Cadenza CD-C18HT analytical column (50x2.0mm, 3 µm) with 98% methanol in 10mM NH_4OAc and 0.1% acetic acid for 1.5 min at 0.6 mL/min. The signal is detected by an IONICS 3Q 100 Series dual probe ion source triple quadrupole coupled to two Shimadzu Prominence UPLC systems (each LC is equipped with a set of pretreament and analytical columns). The total LC cycle time for an injection is 2 min and the sample analysis time is 1 min with alternating injections from each LC. All the solvents used in this method are HPLC grade.

Results

This method covers a concentration range of three orders of magnitude from 0.2 to 200 ng/mL for Tacrolimus, Sirolimus, Everolimus and 2 to 2000 ng/mL for Cyclosporin A, while maintaining good linearity ($R^2 = 0.998$). The intraday and interday CVs for three levels QCs were all $< 9\%$ and $< 11\%$, respectively. No interference or cross contamination was observed.

Conclusion

A sensitive, reliable and accurate LC-MS/MS method was developed and validated for quantification of Tacrolimus, Sirolimus, Everolimus and Cyclosporin A in whole blood. The use of novel dual electrospray source multiplexing technology allows a sample analysis time of only one minute, which doubles the throughput. This LC-MS/MS method requires simple sample preparation and is well-suited for routine therapeutic drug monitoring of immunosuppressive drugs.

PMo-165
11:10 – 12:20

Evaluation of Pt-deposited Porous Alumina Target Plate with MALDI Spiral-TOFMS

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Keywords:
SALDI, Matrix free, MALDI, Spiral-TOFMS, Mycolic acid

Novel aspects:
Pt-deposited Porous alumina plate as a target plate of SALDI MS was developed. Combination of Pt-deposited porous alumina target plate and MALDI Spiral-TOFMS is allowing easy identification of mycolic acids.

Abstract:

Introduction

MALDI-MS came into wider use for biological samples. However, low molecular weight compounds including lipids are difficult to be analyzed by MALDI-TOFMS, because many intense peaks derived from chemical matrix appear in the low mass region.

We have developed a surface-assisted laser desorption/ionization (SALDI) target plate made of Pt-deposited porous alumina surface. The pore dimensions were 100-200 nm in diameter and ca. 500 nm in depth and Pt thickness is less than 50nm. As porous alumina target plate does not require matrix, the resulting mass spectra are clean in the low mass region, and sample preparation is easier than that for MALDI.

In this study, mycolic acid methyl esters were analyzed by Pt-deposited porous alumina target plate for evaluation of its suitability for the SALDI platform.

Experimental

Mycolic acids were extracted from actinomycetes (*Rhodococcus erythropolis* NBRC100887, *R. erythropolis* NBRC13914, *R. equi* NBRC101255T, *Gordonia amarae* NBRC15530T) and they were methylesterified. Each sample was dissolved in tetrahydrofuran (THF), and sodium iodide (NaI), a cationizing reagent, was dissolved in THF at 1 mg/ml. For sample preparation, the NaI solution was deposited on a porous alumina target plate, and then overlaid with sample solution on the plate. In case of conventional MALDI measurement, 2,5-dihydroxybenzoic acid as a matrix compound was overlaid with sample and NaI solutions on a stainless target plate.

MALDI Spiral-TOFMS (JMS-S3000 : JEOL) equipped with Nd : YLF laser (UV : 349nm) was used for SALDI MS and MALDI MS. The effective flight length of Spiral-TOFMS is ca. 17m, and over 60,000 of resolving power (m/Δm : FWHM) is obtained at *m/z* 2,500. Acquired mass spectra were analyzed by Polymerix (Sierra Analytics, Modesto, CA) software and a commercially available spread sheet software.

Results

In both of SALDI and MALDI mass spectra, the sodium-adduct ions of mycolic acid methyl esters were observed in a *m/z* 400-900 region where five different species of mycolic acid molecules were identified. Both mass spectra were similar each other with respect to the relative intensities of the mycolic acid ions. However, the peaks derived from the matrix compound were not observed in the SALDI mass spectrum, allowing easy identification and assignment of the ions especially in the low mass region compared with MALDI mass spectrum. These results indicate that the Pt-deposited porous alumina target plate is useful as a sample target of SALDI MS.

Acknowledgement

The work was partly funded by a grant from the Institute for Fermentation, Osaka (IFO).

PMo-166
13:30 – 14:40

New Version of Desorption Corona Beam Ionization Source and Applications

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Keywords:
DCBI；ionization source；direct analysis

Novel aspects:
DCBI source is capable of direct analysis without sample pretreatment.

Abstract:
A Desorption Corona Beam Ionization (DCBI) source has been reported previously with its capability of providing an alternative direct analysis method mainly for analytes with weak polarity and low mass. In the current version of our DCBI source, a number of new features were introduced while maintaining helium as discharge gas for forming the visual corona beam which serves desorbing and ionizing samples from surface. A new set of DCBI source was developed and it contains a newly designed DCBI probe and its control module. The thin wall stainless steel tubing used for gas heating is effective for fast temperature variation and the desorption temperature the probe can reach is beyond 375°C at its maximum flow rate of 2 L/min. Due to the high desorption temperature, molecules with mass up to 1544 dalton have been detected successfully. For implementing high throughput and reproducible analysis, automated sampler as well as manual sampler has been developed. Some features of the DCBI source including thermal stability of the probe (+/- 5 &#8451；, maximum 375&#8451；) , sensitivity (hexyl cinnamaldehyde 1 pg, S/N= 6 ： 1 at m/z 217, scan mode) , reproducibility (RSD= 8 % (n= 4)) and dynamic range (5 orders) have been obtained. With this improved version, the DCBI source was further tested in the fields of food/drug safety and environmental monitoring. Results will be shown for the analysis of compounds in complex matrices such as herbal medicine, blood and urine. Data for combining this DCBI source with some newly developed sample preparation techniques (such as Frontal Elution Paper Chromatography and Micropipette Tip-based Micro-extraction) will also be presented for the purpose of furthering reducing the effect of matrices.

PMo-167
11:10 – 12:20

Development of TLC/MS by LI-API and WAr-APPI (Photoionization in Argon Gas Contained Water) Method

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Keywords:
Thin Layer Chromatography, TLC/MS, Atmospheric Pressure Ionization, Photoionization, Penning Ionization

Novel aspects:
A photoionization method with the UV lamp which we developed was performed in argon gas soaked with water. and, New TLC/MS using this method and LI method were developed.

Abstract:
A WAr-APPI method, ionization with low-pressure mercury lamp which installed in flow of moist argon gas, has been found by me. This method is one of a photoionization different from penning ionization of direct analysis in real time (DART) . A penning ionization (LI-API) method of the liquid developed by Tuchiya which does not contain water in gas was also considered. As a simple separation method and a detection for sample, thin layer chromatography (TLC) is widely used like column chromatograph even now. In mass spectrometry of TLC, TLC/MS using desorption electrospray ionization (DESI) , DART, and matrix-assisted laser desorption/ionization (MALDI) as a detecting element will be in use. They, WAr-APPI and the LI-API, were discussed for ion source of the TLC/mass spectrometry. Additionally, we developed desorption method of sample which developed on thin layer (TL) plate and the method of ionizing efficiently. They were carried out by the following methods. TL plate was intercalated in the portion which processed to concave state a tip of the tungsten pipe. And, hot argon gas that controlled temperature was sent from lower side of pipe. A vaporized sample detached from TL plate was ionized by different two ionization methods used under atmospheric pressure. In the case of a put sample on sample holder, in ionizing of peroxides sensitive with heat by LP-API, although it is the ionizing method excellent in the usual sample, No protonated molecule ([M+H] +) of their sample could be observed. However, it was solved by using an addition reagent. On the other hand, by the WAr-APPI method, the [M+H] + of peroxide such as t-butyl peroxy laurate appeared without decomposition. The reason why the peroxide sensitive with heat does not disintegrate will be that water acted as a decomposition suppressant. To this verification, the effect of the added water in argon gas and the appearance state of the fragment ion which occurs with heat was investigated. With the increase in water, the fragment ion of peroxide generated with heat disappeared, and no [M+H] + was observed in case of dry argon gas. Therefore, it seemed that this method was good to detect ion of the peroxide. As for helium gas, the effect was smaller than that of argon gas. No nitrogen gas was observed. At TLC, or desorption from TL plate of sample, the ionization was performed to both LI-API and WAr-APPI. Both solid methyl stearate and liquid methyl laurate at the room temperature which used as a primary standard sample presented the signal of the beautiful [M+H] +. The limit of detection of methyl stearate by LI-API method was 6 ng which is enough to actually use. This fact shows that desorption of sample and ionization were fully performed. A new method of desorption of sample on TL plate was a method which is heated from the two side of the surface of the plate, unlike the reflective method on the plate of DART or desorption electrosplay ionization (DESI) . Therefore, it is thought that sample detached with this method from TL plate efficiently. Furthermore, as dimethyl sulfoxide (DMSO) solvent is difficult for MALDI/MS, WAr-APPI also examined. A [M+H] + of the synthesized sample was clearly observed without the influence of the solvent. As for LI-API, the influence of various solvents such as DMSO, tetramethylsilane) (TMS) , and tetrahydrofuran (THF) was investigated similarly. The analytes were used the synthesized sample with the functional group (such as hydroxy, oxo, double bond, and bromide) which may be affected by heat. We were satisfied with the obtained result. In addition, Ni complex has also been measured on a par with fast atom bombardment (FAB) used for metal complex.

PMo-168 Charged-droplet beam source using vacuum electrospray of an ionic liquid for secondary ion mass spectrometry (SIMS)

13:30 – 14:40

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AIST, Tsukuba, Japan

Keywords:
vacuum electrospray, charged droplet, ionic liquid, ion beam, cluster, SIMS

Novel aspects:
Obtained results are important to develop a primary ion beam source for SIMS, which is capable of producing massive ions at higher current density with a smaller beam spot size.

Abstract:
Secondary ion mass spectrometry (SIMS) is a powerful technique for characterizing the surface and interface compositions of inorganic and organic materials. Polyatomic (or cluster) primary ion beams can greatly increase yields of higher-molecular-weight secondary ions, enabling molecular imaging of organic and biomolecules. [1] For these reasons, cluster ions, such as Au_3+, C_{60}+, $Ir_4(CO)_7$+, Ar_n+ and charged droplets $[(H_2O)_{90,000}+100H]^{100+}$, have been used in recent years. [1-4]

For further improvement in SIMS analysis, it is required to develop a new cluster ion source capable of producing massive ions at higher current density with a smaller beam spot size. From the viewpoint of generating such massive ions, vacuum electrospray of ionic liquids is expected to have a great potential ; for instance, it probably improves beam-transport efficiency, thereby increasing current density as well as beam current [5-7] .

Ionic liquids $[C^+A^-]$ represent room-temperature molten salts consisting of a cation $[C^+]$ and an anion $[A^-]$. Since they have a negligible vapor pressure and a high ionic conductivity, it is possible to electrospray them in a vacuum. A wide variety of ionic liquids has been synthesized commercially in recent years. Among these, we tested a quaternary ammonium ionic liquid, N,N-diethyl-N-methyl-N- (2-methoxyethyl) ammonium bis (trifluoromethanesulfonyl) amide (DEME-TFSA) . [8]

To develop a vacuum-electrospray beam source for SIMS, we investigated beam characteristics of charged particles electrosprayed in vacuum in negative-ion mode as well as positive-ion mode. [9,10] In terms of practical use as a primary ion beam source in SIMS, several factors have to be taken into account ; e.g., (i) beam current, (ii) efficiency of beam generation, and (iii) distributions of mass and charge of emitted droplets. For these reasons, we have investigated such factors under various experimental conditions [8-11] . Obtained results indicate that vacuum electrospray of ionic liquids is applicable to a massive-cluster beam source for SIMS.

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PMo-169
11:10 – 12:20

High Throughput Molecular Weight Confirmation of Pharmaceutical Compounds Using DART MS Analysis with Ultra-fast Polarity Switching

Teruhisa Shiota[1], Hirotaka Honda[1], Jun Watanabe[2], Kazuo Mukaibatake[2], Junko Iida[2], Tsubasa Ibushi[3], Manabu Ueda[2]

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Keywords:
Ultra-fast Polarity Switching, Direct Analysis in Real Time, Pharmaceutical Compounds, DMSO

Novel aspects:
A high throughput analysis method for pharmaceutical compounds dissolved in non-LC/MS compatible solvent utilizing the ultra-fast polarity switching was developed.

Abstract:

Introduction

DART, a direct atmospheric pressure ionization source, is known to have little or no negative effect from the solvent used to dissolve the sample. Even with problematic solvents for LC/MS operation such as non-volatile solvents or solvents containing salt, the target compounds can be instantly identified using DART-MS without any sample preparation. High-throughput MW confirmation of synthesized compounds is difficult to achieve using time of flight mass spectrometers, which have been a common choice to interface DART, due to its limitation in switching between positive and negative ion modes.
By combining an automated DART ion source with a quadrupole mass spectrometer with ultra-fast polarity switching capability, 11 different pharmaceutical compounds with various polarities were successfully determined in approximately 10 sec/sample.

Method

DART-MS analyses of 10ppm pharmaceutical compounds dissolved in 100 % DMSO were carried out. The DART-SVP ion source (IonSense, MA, USA) was coupled to the single quadrupole LCMS-2020 (Shimadzu, Kyoto, Japan) , and ID CUBE (IonSense) , the new type of DART, was also coupled to the said mass spectrometer. Introduction of samples for DART-SVP was automated by using the X-Z scanner, which is capable of automating up to 96 samples/run. Ultra-fast polarity switching was utilized on the mass spectrometer to collect full scan data. LCMS-2020 can achieve the polarity switching time of 15msec and the scanning speed of up to 15000u/sec, therefore the loop time can be set at less than 1 second despite the relatively large scanning range of 100-1000u.

Preliminary Data

First, the new ID CUBE ion source was tested and successfully interfaced to LCMS-2020. Mass spectrum was identified for both the positive standard sample of 100ppm quinine and the negative standard sample of 100ppm methylparaben. 5 uL of samples were spotted on a specialized sampling card and inserted into the ID CUBE source. Vaporization of sample is more rapid with ID CUBE than it is with DART since the sample is directly heated with electric current running through the metal mesh on which the sample is applied, while the DART heats the sample with heated gas. Next, the DART gas (helium) heater temperature was raised to 350C and commercially available pharmaceutical compounds such as Atenolol, Warfarin, Yohimbine, Cilostazol, Nifedipine, Diazepam, Nitrendipine, and Diphenhydramine were applied on a metal mesh of the X-Z tool, which has the same size and configuration as the 96 well microtiter plate, to automatically introduce them into the DART ionization gas. Relatively large DMSO peak was observed in positive mode scan, but the target compounds were successfully identified as well. Positive ion spectrum was observed for Atenolol, Diazepam and Diphenhydramine. Negative ion spectrum was observed for Nifedipine and Nitrendipine. Both positive and negative ion spectra were observed for Warfarin, Yohimbine and Cilostazol. DART-MS with the ultra-fast polarity switching and ultra-fast scanning demonstrated its ability to perform high throughput analysis of pharmaceutical compounds dissolved in high concentration DMSO at approximately 10sec/sample.

PMo-170
13:30 – 14:40

Componential Analysis of Pepper of Various Origins Using DART-MS Using Ultra-fast Polarity Switching

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Keywords:
Flavor, Origin, Multivariable Analysis, Ultra-fast Polarity Switching, Direct Analysis in Real Time

Novel aspects:
Rapid DART analysis of food samples using ultra-fast polarity switching to conduct multivariable analysis to distinguish their origins.

Abstract:
Introduction
DART (Direct Analysis in Real Time) , a direct atmospheric pressure ionization source, is capable of analyzing food samples with little or no sample preparation. Time of flight mass spectrometers, which have been a common choice to interface DART, were not suitable in carrying out simultaneous detection of compounds with different polarities from the same sample due to its limitation in switching between positive and negative ion modes in terms of time required to do so,.
Analysis of different parts of pepper (exodermis, endodermis and seeds) along with olive oil and soy source of different types and origins were carried out using the DART combined with a mass spectrometer with ultra-fast polarity switching capability to conduct multivariable analysis.

Method
Commercially available peppers from different origins were introduced to the DART gas using tweezers. Glass capillary was used to dip and transfer olive oil to the ionization area, and soy source was spotted on a metal mesh to scan with the DART beam. The DART (IonSense, MA, USA) was interfaced onto the single quadrupole mass spectrometer LCMS-2020 (Shimadzu, Kyoto Japan) . Ultra-fast polarity switching was utilized on the mass spectrometer to collect full scan data. LCMS-2020 can achieve the polarity switching time of 15msec and the scanning speed of up to 15000u/sec, therefore the loop time can be set at less than 1 second despite the relatively large scanning range of 100-1000u.

Preliminary Data
Capsaicin (C18H27NO3, MW 305) , which is responsible for the spicy flavor of pepper, is usually most abundant in placenta. Commercially available peppers from Korea, China and several areas in Japan were cut and separated to each part of the exodermis, endodermis and the seed and each sample was analyzed by DART-MS. The signal indicating capsaicin was detected in both polarities ; at m/z 306 in positive ion mode, m/z 304 in negative mode. After careful optimization of analytical conditions using different DART gas heater temperatures at 200C, 350C and 500C, it was determined that 350C was the suitable temperature setting to analyze capsaicin. In most of the samples, the signal of capsaicin was most intense in seeds, which implicates that there were placental fragments on the surface of the seeds, which, even in small quantity, was giving out the intense signal for capsaicin.
Next, soy sources and fish sources from different origins were analyzed. Low molecular weight spectra were observed in different profiles depending on the sample ingredients, indicating the different balances in amino acid contents.
For olive oil and grape seeds oil samples, high molecular weight spectra were more dominant in both polarity at temperature setting of 500C than 200C and 350C, suggesting the possibility to analyze the differences in triglycerides contents.
Multivariable analyses were conducted using these spectral data to distinguish different food samples from different origins and/or deferent ingredients. This indicates that DART-MS can be a powerful tool for origin determination/authentication of agricultural produce and processed goods.

PMo-171
11:10 – 12:20

Gas Chromatography/Ambient Ionization Mass Spectrometry (GC/AMS) for the Analysis of Long Chain n-Alkanes in Crude Oil

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Keywords:
Ambient Ionization Mass Spectrometry ; Gas Chromatography ; Alkanes ; Crude Oil

Novel aspects:
Gas chromatography/ambient ionization mass spectrometry was demonstrated to analyze long chain n-alkanes in crude oil.

Abstract:
Gas chromatography (GC) is a well established analytical tool commonly used to separate organic mixtures. The analytes eluted from GC were ionized by electron impact ionization, chemical ionization, electrospray ionization (ESI) , or atmospheric pressure chemical ionization (APCI) (also referred as corona discharging mechanisms) . In this study, we combined gas chromatography with ambient mass spectrometry (AMS) using a plasma based-APCI as the ionization source and interface. The interface of GC-AMS consists of a heated metal tube inserted in a stainless steel chamber and a plasma based-APCI ion source. The ion source is a glass tube covered by a small stainless steel tube as the counter electrode and a ring electrode. As helium stream flows through the APCI source, plasma is generated between the two electrodes.
For a typical analysis, samples were injected in gas chromatography for separation. The separated analytes were carried through the heated stainless steel tube at high temperature, and flowed into the plasma based-APCI source. The analytes were ionized by interacting with the helium plasma species, and subsequently detected by a Q-TOF mass analyzer. For example, a mixture of long chain n-alkanes (n-C_8 to n-C_{34}) and fatty acid methyl esters (FAMEs) standards were prepared and used to examine the performance of the GC/AMS. As it was expected, protonated molecular ions $(M+H)^+$ were detected for all FAMEs. Since the molecules of long chain n-alkanes contain no functional groups to receive proton during ion-molecule reactions, no protonated molecular ions was detected. Instead, nitrogen adduct ions $[(M+N-H)^+]$ were detected for all n-alkanes.

PMo-172
13:30 – 14:40

A Combined DSMC - CFD Approach for the Numerical Simulation of an Under-expanded Jet

Athanasios Zacharos[2], Ioannis Nikolos[3], Dimitris Papanastasiou[2], Roger Giles[1], Andrew Entwistle[1], Emmanuel Raptakis[2]

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Keywords:
DSMC, Under-expanded Jet, CFD

Novel aspects:
A new method for calculating the free jet expansion region with greater precision has been developed. A combined approach using DSMC and CFD is employed.

Abstract:
A combined Direct Simulation Monte Carlo (DSMC) Computational Fluid Dynamics (CFD) approach is adopted to calculate the free jet developed through a long capillary inlet at the fore vacuum of a mass spectrometer. The transition from the continuum to the rarefied flow regime requires different solvers to be employed in order to describe the free jet with greater precision. The simulation domain is divided into two regions, the continuum regime where the CFD method can be applied successfully and the molecular flow regime where the DSMC method is well known to provide more physically accurate results. The combined solution is compared with the one obtained by applying the Navier-Stokes equations across the entire domain.
The CFD solver is first applied across the entire domain, which includes the atmospheric pressure region connected to the fore vacuum region through a 100 mm long 0.5 mm ID capillary. Nitrogen pressure in the vacuum region is set to 20 mbar. The DSMC solver is applied across the last 2 mm of the capillary and throughout the low pressure region. The DSMC boundary conditions at the inlet of the domain are determined by the CFD calculations. The DSMC code has been modified to accept the parabolic velocity profile of the gas flow near the capillary exit. Salome™ open-source software is used for the generation of the unstructured mesh and computations are performed in OpenFOAM™. The DSMC simulations are performed on the grid computer system of the Technical University of Crete, comprising 44 HP Proliant BL465c server blades with two AMD Opteron Model 2218 processors each.
The CFD solution for the nitrogen free jet expansion at 20 mbar shows the formation of a barrel shock, the presence of a Mach disk formed a few mm downstream the capillary outlet followed by the silent zone. Oscillations of the axial velocity observed further downstream are attributed to shock waves of decreasing strength. The onset of turbulence at the jet breaking point is also clearly observed including the region where the flow becomes subsonic. The region where CFD is expected to provide results with greater accuracy is the flow across the capillary where the drop in pressure is accompanied by a significant acceleration of the gas toward the outlet. No slip conditions are imposed across the length of the capillary. In contrast, the DSMC solver shows that slip velocity conditions develop near the outlet despite the short distance allowed for the gas to accelerate near the capillary outlet. The two solutions are compared in terms of the structure of the barrel shock, the Mach disk, the axial velocity oscillations and radial velocity profiles. The turbulence and subsonic regions developed deep inside the vacuum region where the DSMC solution is expected to give more accurate results are also investigated in greater detail. The discrepancies and limitation of the DSMC and CFD methods with respect to their applicability on the free jet theme and with particular interest on the design of mass spectrometers are highlighted.

PMo-173 Proposal of the New Method for 3DAP Analysis of Biological Molecules Utilizing Photocatalyst and Mesoporous Materials

11:10 – 12:20

Masato Morita, Mahito Shimizu, Yuya Hanaoka, Masanori Owari

The University of Tokyo, Tokyo, Japan

Keywords:
3 DAP, Photocatalyst, Biological molecules, Mesoporous, Laser

Novel aspects:
The application of photocatalyst to the field evaporation for analyzing covalent materials in the 3 DAP.

Abstract:
The three dimensional atom probe (3 DAP) is one of the most unique three dimensional imaging technique which has high spatial resolution and high detection sensitivity. The basic principle of the atom probe is based on field evaporation of surface atoms in the needle whose apex is typically 100 nm in radius. These atoms are chemically mass indentified by time-of-flight. Three dimensional position of each atom is calculated from the coordinates on the position sensitive detector and the sequence of detection. From such information, the three dimensional structure of the sample can be observed with atomic scale. However, the analysis of covalent materials such as organic and bio molecules has not succeeded. If the 3 DAP analysis of organic material is possible, many contributions to various fields are expected. In particular, the analysis of biological specimen may dramatically advance researches of medicine and pharmacy.

During the 3 DAP analysis, a high voltage is applied to the sample. This results in a very strong electric field more than 10 V/nm at the apex of the sample allowing ions to field-evaporate from the surface. The strong electric field induces mechanical forces (electric stress) at the sample, which eventually lead to the rupture of the sample. Therefore, a certain amount of conductivity and mechanical strength are needed for the sample of 3 DAP analysis. From the beginning, 3 DAP specializes to the analysis of high electric conductivity materials such as alloy. In recent years, by application of the femto second pulsed laser, 3 DAP has many good contributions in the analysis of semiconductor-based electronics. However, in the case of analysis of covalent materials, there are some difficulties : fragment ions which have various sizes are generated at field evaporation, sample fabrication by focused ion beam (FIB) is very difficult, a rupture of sample by high electric stress frequency occurs, and so on. The field evaporation of carbon atom requires very high electric field (103 V/nm) compared to metallic atom. Therefore, a higher voltage is applied to the organic sample and a higher electric stress causes the rupture of sample earlier than field evaporation. On the other hand, the fragment ions complicate the mass spectrum and worsen the spatial resolution. To resolve these problems, application of the short wavelength pulsed laser for the 3 DAP was proposed. However, the short wave length pulsed laser is very expensive and causes the low output power. Since the photon energy assists the ionization, the adjustment of wavelength is needed depending on the materials. Moreover, alignment between the sample apex and laser irradiation position is very difficult with invisible wavelength.

In this study, we proposed the new ionization process for 3 DAP analysis of organic materials utilizing the photocatalyst. This method enables to make organic materials ionize with lower voltage and the conventional wavelength laser. The adjustment of wavelength is not required depending on the materials. It is expected that the extent of fragmentation is controlled and the mass spectrum are not complicated. In this study, we evaluated the effect on the mass spectrum in the case of the new ionization process utilizing the photocatalyst. Another problem to be resolved is the sample fabrication. The 3 DAP sample was prepared utilizing mesoporous materials. The mesoporous material was designed with photocatalyst material and an organic material was enclosed in pore. Since a frame is an inorganic material, the needlelike sample fabrication by FIB is possible. Moreover, the sample of covalent material is supplemented with a certain amount of conductivity and mechanical strength.

PMo-174
13:30 – 14:40

High Sensitivity Spray Ionization Approaches at AP and Vacuum with Matrices or Solution With or Without Voltage or a Laser

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Keywords:
Spray ionization, inlet ionization, MALDI, electrospray ionization, mechanism

Novel aspects:
Several new ionization approaches are presented that produce multiply charged ions from solution or a solid matrix, from atmospheric pressure or vacuum, with or without a laser or voltage.

Abstract:
In mass spectrometry 'spray ionization', means electrospray ionization (ESI) , but in nature charged droplets or particles are common, being produced from an ocean spray or even as a spray of charged ice particles from a freezing water droplet. Producing ions from sprayed solutions for mass spectrometry has been accomplished using ESI, sonic spray ionization, thermospray ionization, and more recently we introduced solvent assisted inlet ionization. There are also techniques such as fast atom bombardment which may produce ions by 'microspray' ionization . The commonality of these methods, operating at AP or vacuum, are that the ions observed using mass spectrometry are produced from charged droplets similar to ESI, but without applying a high voltage. We will demonstrate a highly sensitive solution spray ionization approach that uses a combination of methods in which the ionization selectivity (or suppression) can be switched to favor different classes of compounds.

'Spray ionization' is also applicable to ionization from solid matrices. Recently, laserspray ionization, a method operationally nearly identical to AP-MALDI, was shown to produce multiply charged ESI-like ions from solid matrices commonly used in MALDI. However, the laser was not necessary for ion production, and simply introducing the matrix analyte into a heated inlet tube linking AP and the first vacuum region of a mass spectrometer produced nearly identical ESI-like ions. This approach was extended to produce multiply charged ions using a vacuum MALDI source. We will provide data supporting the conclusion that solid matrices, acting as solutions, produce ions by a form of spray ionization. Again, neither a laser nor a voltage is required, and we demonstrate this by ionizing proteins directly from ice. The data provided by these experiments show that photochemical ionization is not necessary for producing ions from proteins as large as BSA under MALDI conditions.

We will present new highly sensitive ionization methods, producing ions from solution or solid matrix introduced to the mass analyzer from atmospheric pressure or directly into the vacuum, with or without use of a laser or voltage for ion production. The mechanistic implications of these studies for the ionization of nonvolatile compounds will be related to a variety of ionization approaches used in mass spectrometry.

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PMo-175
11:10 – 12:20

Fundamental Aspects of Solvent free Atmospheric Pressure Chemical Ionization and Laser Diode Thermal Desorption Ionization Source (LDTD)

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Keywords:
Desorption, Thermal ; Ionization, Atmospheric Pressure Chemical ; Mass Spectrometry

Novel aspects:
New insights in thermal desorption process involved in LDTD-APCI source are introduced in terms of enthalpy of volatilization, thermodynamics and solvent less APCI.

Abstract:

Introduction

The Laser Diode Thermal Desorption (LDTD) ionization source allows the analysis by mass spectrometry of samples at atmospheric pressure, minimizing preparation steps. The LDTD uses a Laser Diode in the transmission geometry to produce a rapid thermal desorption of the sample deposited in a dedicated 96 well holder followed by atmospheric pressure chemical ionization. The purpose of this work is to present the measurements of the main characteristics of the source and the associated thermodynamics behavior.

Methods

A LDTD source was modified to monitor the temperature on the 96 well sample holder during a real-time thermal desorption experiment. Different combinations of thermal desorption profile and carrier gas flow have been used to monitor the sample holder surface temperature produced by the laser diode radiation. The thermal desorption of sulfadiazine in function of the surface temperature was measured while the influence of the carrier gas flow on the signal intensity was evaluated using clomiphene. Thermodynamic theory is applied to corroborate the experimental data and electron microscopy is used to characterize the sample crystallization on the surface of the well. All the experimental work has been performed on a LDTD model T-960 interfaced to a TSQ Quantum Ultra AM.

Preliminary results

The gas flow applied to carry the vaporized neutral sample into the APCI region for ionization influences the signal for clomiphene. Maximum signal is obtained with a carrier gas flow set between 2.0 and 3.5 L/min. At a lower or higher carrier gas flow, the signal intensity decreases and the variability increases. The intensity of the signal as a function of temperature for Sulfadiazine gives the behavior of the volatilization process, showing beginning of the process at 60° C up to a maximum starting at 95 ° C and stable until 140 ° C is attained. The influence of the temperature sensor on the measurement is evaluated to be 14 % Computed temperature for the incident power shows good agreement with measured values at 200 ° C. Deposit dimension measured with the electron microscope on the surface are in the nanometer range as expected. The theory of the crystallization at the nanoscale can then be applied to explain the lower value of volatilization enthalpy observed on compounds that should decompose before melting as in the bulk experiment. Characterization of the APCI by monitoring the water concentration and solvent addition show proton affinity effect. Optimums are confirmed by the protonation of a fully saturated fatty acid. Flow of available protons produced by the corona discharge should be in theory at 10^{-10} Mol/sec. Binary mixture of compounds corroborate showing 50% suppression of the signal at $3.8x10^{-10}$ Mol vaporized.

PMo-176
13:30 – 14:40

Photo-ionisation mass spectrometry for on-line monitoring: Technology and real-time monitoring applications such as characterisation of the coffee-roasting and cigarette-smoking process

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Keywords:
photo ionisation, on-line real-time measurement, coffee-roasting, cigratte smoking

Novel aspects:
New application of on-line photo ionisation-MS, Setup of optimised VUV-light sources, On-line-analysis of roasting-degree and flavour formation-mechanisms (coffee) as well as pyrolysis chemistry and formation-mechanisms of hazardous chemicals (cigarette)

Abstract:
In the last decade, several soft ionisation **M**ass **S**pectrometric (MS) techniques for on-line monitoring of complex gas mixtures were developed. This included **C**hemical **I**onisation techniques as well as photo ionisation (PI) based mass spectrometric technologies. **P**hoto **I**onisation mass spectrometry is particularly well suited to monitor the chemical signatures in combustion and pyrolysis processes. On the one hand, the laser based **R**esonance **E**nhanced **M**ulti-**P**hoton **I**onisation technique (REMPI) allows the ultra-sensitive and selective detection of aromatic substances in complex gases ; on the other hand the **S**ingle **P**hoton **I**onisation method (SPI, with VUV-photons) features an overview characterisation of all organic compounds. Several photo-ionisation mass spectrometric application e.g. for on-line monitoring of puff-resolved tobacco smoke [1] , waste incineration flue gas or pyrolysis processes have been reported. In an on-line analysis study of the coffee-roasting process it was demonstrated that a prediction of the degree of roasting from the chemical signature of the roasting-off gas is feasible by laser ionisation mass spectrometry (SPI/REMPI) [2] . Recently also a less sophisticated SPI mass spectrometric photo-ionisation method, based on a novel lamp-like VUV-light-source (**e**lectron **b**eam pumped rare gas **e**xcimer **l**ight-source, EBEL) , has been developed [1] . The EBEL-SPI technology allows fast real-time mass spectrometric analysis of organic mixtures. Depending on the used wavelength (i.e. type of rare gas filling of the EBEL) all organic substances are accessible including alkanes, alkenes, naphthenes, aromatic compounds, carbonyl-compounds or heterocyclic species. Detection limits in the low ppb concentration range are achieved at a time-resolution of some seconds. The novel EBEL-SPI technique up to now has been used in conjunction with different mass spectrometric technologies (**Q**uadruple- (QMS) , **T**ime-**O**f-**F**light- (TOF-MS) and **I**on **T**rap-mass spectrometers (ITMS)) . The EBEL-SPI-ITMS approach has advantages for direct analysis of complex mixtures such as coffee roasting gases as a direct confirmation of the recorded analyte is possible by tandem-MS [3] . Several practical applications of the SPI-MS technology with EBEL-VUV-light-sources (and laser based REMPI/SPI) are discussed. This includes the analysis of the coffee roasting process, the puff-resolved detection of chemicals in tobacco smoke as well as field-applications for process-monitoring (on-site-analysis) .
For the coffee-roasting application in addition to the measurement of the bulk gases also a newly developed µ-probe sampling technique was applied. This approach allows the monitoring of the formed roasting gases directly inside individual coffee beans : A hole of ~400 µm diameter was drilled in the green coffee bean and the 300µm diameter stainless steel tip of the µ-probe was inserted in the hole, which was sealed thereafter with inorganic glue. Subsequently the individual bean was hot air-roasted at 200 °C and the evolved gases from the inside of the investigated bean were directly sampled into the PI-TOFMS. During the roasting process, a variety of organic compounds are sensitively detectable. The on-line detected chemical signature comprises a variety of compounds and compound classes including phenols, sulphur-compounds, carboxylic-compounds, nitrogen-compounds, furan-derivatives, fatty-acids and of course caffeine. Typical time-intensity profiles were observed and different compounds have also a different precursor behaviour and react also differently on the rupture of the cellular structure of the coffee-bean during roasting (cracking, caused by the CO_2 formation due to decarboxylation of ferula acid) . Measurements of several individual bean roasting-profiles from the Robusta and Arabica coffee varieties have been statistically analysed. Markers for roast-degree and variety-type have been identified. The µ-probe also was applied to analyse the chemical compounds in the pyrolysis- and combustion-zones within a cigarette during puffing.
Additionally on-line process analysis results at a biomass-to-liquid plant are discussed.

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PMo-177 Design of Electrodynamic Ion Funnel-Stacked Ring Trap

11:10 – 12:20

Liulin Deng, T-W Dominic Chan

The Chinese University of Hongkong, Hongkong

Keywords:
ion funnel, ion funnel trap, ion funnel-stacked ring trap

Novel aspects:
IF-SRT is consisted of ion funnel and stacked ring trap

Abstract:
Electrodynamic ion funnel (IF) is a device developed by Shaffer and co-workers[1-3] for transmission of ions across the medium pressure region (i.e. 1 30 torr) of typical atmospheric ionization sources. This device can transmit ions with wide spatial and energy dispersions and focus them into a narrow ion beam. It was designed to replace the conventional skimmer electrode to improve the ion transmission efficiency while maintaining the conductance limiting function. Electrodynamic ion funnel trap (IFT) was proposed by Ibrahim and co-workers[4] for accumulation of ions behind pre-IF and followed by post-IF which can extract and refocus all trapped ions to down-stream region, high trapping efficiency can improve sensitivity of IMS and MS instruments. As part of the instrumental rejuvenation program, a 100-plate electrodynamic ion funnel was designed and fabricated in house to improve the performance of the 4.7 Tesla FTMS instrument. Moreover, electrodynamic ion funnel-stacked ring trap (IF-SRT) we proposed here has a different structure compared to IFT, it has two main sections : the first section is a IF which consists 66-plate to capture and focuses ions with high transmission efficiency ; the second section has 34 constant internal diameter (3.0mm) electrode rings, effective potential in radial direction is generated by applying RF potentials with opposite phases in adjacent electrode rings, different DC gradient potentials by adjusting a pulse voltage applied to the last electrode are utilized in axis direction to control ions working in transmit (+ 5 V/cm) or trap (- 5 V/cm) mode. We have constructed IF-SRT model in SIMION 8.0 software and investigated ion transmission and trapping performance with different DC potentials of IF-SRT.

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PMo-178
13:30 – 14:40

Delayed electron emission and radiative cooling of stored molecular ions in TMU electrostatic ion storage ring

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Keywords:
electrostatic ion storage ring, laser excitation, cooling process, slow dynamics

Novel aspects:
An electrostatic ion storage ring allows us to keep molecular ions in vacuum for a period up to a few minutes to study their slow dynamics by combination of laser-excitation.

Abstract:
An electrostatic ion storage ring allows us to keep molecular ions circulating in vacuum for a long period up to a few minutes to study their slow dynamics. Since this type of electrostatic rings is free from restriction of the ion mass, large polyatomic molecular ions can be stored.

By introducing a visible tunable laser, we investigated the evolutional change of the delayed electron emission from negative ions depending on the ion storage time, and succeeded in extracting the dynamics of the de-excitation process of the hot molecular ions through delayed electron emission and IR radiation.

One example is determination of the cooling rate and the internal temperature of hot large polyatomic ions. The cooling rates of hot C_{60}^- have been measured in the time region up to several tens of ms by observing the delayed electron detachment yield with one-photon laser excitation. The absolute energy scale is established by the photon energy and the cooling time interval is derived from the non-exponential statistical decay of the ensemble of hot molecules.

Another typical example is a small molecular ion. One-photon laser induced decays have been measured for C_5^- ions with small heat capacity. Photon-induced decays were found to be again statistical and influenced by radiative cooling. We measured the photo-enhanced decay at different storage times and photon energies. The electron detachment yield increased as the storage time advances, and after the maximum, they reversely decreased. This characteristic behavior is well explained by the concept of energy window for electron detachment observation using this ion storage ring, and again allowed determination of the cooling rate. The average internal energy content varies as time to the power of 0.5, which is consistent with a T^4 cooling power.

When the stored ion is a large molecule with a large heat capacity at moderately hot temperatures, these one-photon methods are not applicable due to shortage of the necessary excess energy for electron emission. To heat such targets sufficiently, an alternative scheme with sequential multi-photon laser excitation was also tried for zinc phthalocyanine anions, $C_{32}H_{16}N_8Zn^-$. We extracted the information on the cooling behavior by observing the change in the fluence dependence of the laser induced electron detachment yield.

Thus, by applying a variety of methods suitable for each target ion, we confirmed that the cooling behavior of the molecular ions is well understood by the electrostatic ion storage ring and combined with a laser excitation.

PMo-179 Develapment of a cryogenic ion storage ring in RIKEN

11:10 – 12:20

yoshinori enomoto, yuji nakano, takuya masunaga, toshiyuki azuma

RIKEN, Wako, Japan

Keywords:
cryogenic ion storage ring

Novel aspects:
Our new ion storage ring realize completly cryogenic environment.

Abstract:
We have been developing a new electrostatic ion storage ring in RIKEN.
Compared with storage rings which use magnetic field to control ions, electrostatic ones can control ions regardless of their mass. Thus heavy ions like biomolecule can be stored in the same way as light atomic ions.
First electrostatic ring, ELISA, was developed in 1997 at Aarhus University. Subsequently, similar rings were constructed several places. However, these rings are operated in a room or liquid nitrogen temperature. Stored ions are heated by radiation from electrodes and walls of vacuum chamber. Vibrational or rotational states of them are excited and obtained spectra are convolution of many states. In addition, storage time of ions is limited by collision with residual gas molecules. Although pressure in these rings is about 10^{-11} Torr, typical storage time is only 1 s. Thus it is difficult to observe slow or rare process.
As next generation storage rings to overcome these issues, cryogenic rings, whose environmental temperature is below 10 K, are under development at several institutes in the world.
Features and design objectives of our ring is as follows.
(1) Ultimate environmental temperature is 5 K.
(2) Ultimate pressure in the ring is 10^{-14} Torr.
(3) Liquid He free cooling system.
At present, we finished detailed design of the ring, and it is under construction. We will show details and status of our new ring at the conference.

PMo-180
13:30 – 14:40

Development of MALDI-MS-based imaging technique for visualizing the green tea polyphenol EGCG in mammalian tissues

Yoshinori Fujimura, Yoon Hee Kim, Takatoki Hagihara, Daichi Yukihira, Ayumi Yamaguchi, Miho Irie, Daisuke Miura, Hiroyuki Wariishi, Mitsuru Shindo, Hirofumi Tachibana

Kyushu University, Fukuoka, Japan

Keywords:
MALDI-MS ; Imaging ; Polyphenol ; EGCG ; Mammalian-tissue

Novel aspects:
We succeeded for the first time in developing a novel MALDI-MSI technique for visualizing the green tea polyphenol EGCG on the mammalian tissue section.

Abstract:
Introduction and objective: Epigallocatechin-3-*O*-gallate (EGCG), the major active polyphenol in green tea (*Camellia sinensis* L.), has been shown to possess various health promotion effects. Although the understanding of spatiotemporal distribution in tissues is indispensable for elucidating the detailed mechanism of EGCG action, such information still remains unclear. Mass spectrometry imaging (MSI) is a remarkable new technology that enables us to determine the distribution of endogenous or exogenous molecules present in tissue sections by direct ionization and detection with matrix-assisted laser desorption ionization (MALDI)-MS[1]. Recently, we have developed the highly sensitive MSI technique for visualizing of spatiotemporal dynamics of the tissue metabolome[2]. In this study, we attempted to develop a MALDI-MSI technique for visualizing two-dimensional distribution of EGCG in tissue micro-regions.

Methods and results : We have recently reported that a MALDI-MS system with 9-aminoacridine (9-AA) achieved a great improvement for the sensitivity of low-molecular-weight metabolite analysis that is advantageous for the simultaneous detection of a variety of cellular and tissue metabolites[2-4]. Here we applied this technique for imaging the major green tea polyphenol EGCG with higher sensitivity. First of all, more than 40 kinds of chemical compounds, including 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA), and 9-AA, were examined for screening the potential matrix against EGCG. Spots of mixed solutions of these chemicals and EGCG were measured by MALDI-TOF-MS in both positive and negative ionization modes. An EGCG peak was not observed by the major matrix (DHB, CHCA, SA, and 9-AA). On the other hand, β-carboline derivatives, CHCA analogues, and naphthalene derivatives were able to detect EGCG with higher sensitivity (femtomole order) in negative ionization mode. Almost all matrices capable of detecting polyphenols as previously reported had no ability to detect EGCG with the same sensitivity. To examine whether these chemicals can visualize EGCG on the tissue section, we were undertaken to detect an image of EGCG spotted on the normal mouse liver section. Although MSI experiment revealed that a peak intensity of EGCG was greatly reduced as compared to the case of the screening test on a stainless MALDI sample plate, two-dimensional image of EGCG spot was obtained with signal-to-noise ratio of more than 100.

The principal findings : These results suggest that our selected chemicals act as a matrix against the green tea polyphenol EGCG on both the sample plate and the tissue section. This finding is the first report showing that our proposed MALDI-MSI techniques enable to visualize two-dimensional image of EGCG. Now, we are trying to detect EGCG in the liver section derived from EGCG-administrated mouse.

References : [1] *J. Proteomics*, in press, 2012 ; [2] *Anal. Chem*., **82**, 9789, 2010 ; [3] *Anal. Chem*., **82**, 4278, 2010 ; [4] *Anal. Chem*., **82**, 498, 2010

Acknowledgements : This work was supported in part by Grants for project research (Development of fundamental technology for analysis and evaluation of functional agricultural products and functional foods (to H.T.), and Grants-in-Aid for Scientific Research (S) (Grant 22228002) (to H.T.). We would like to specially thank Shimadzu Corporation. (Kyoto, Japan) for their full cooperations.

PMo-181
11:10 – 12:20

Development of micro-focusing electrospray droplet impact gun for SIMS analysis

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University of Yamanashi, Kofe, Japan

Keywords:
electrospray, electrospray droplet impact (EDI), SIMS

Novel aspects:
A newly designed ambient electrospray droplet impact (EDI) gun could be focused to about 40 micron with 8 kV acceleration voltage with the beam current of 0.5nA.

Abstract:
A noble ionization method, the electrospray droplet impact (EDI), has been developed for cluster SIMS. [1] The projectiles used in EDI are extremely large multiple charged water cluster ions with masses of several 10^6 u. The EDI gun was installed in an orthogonal time of flight mass spectrometer (TOF-MS) (JEOL, Accu-TOF, Japan). The organic, inorganic and semiconductors materials were examined by EDI/SIMS. It was found that the surfaces etched by EDI suffered from neither degradation nor preferential sputtering of particular elements. EDI was found to be capable of molecular level etching without leaving damage after the irradiation. [2], [3]

The charged water droplets were formed in ambient by electrospraying 0.01 M trifluoroacetic acid aqueous solution. The droplets were sampled through an orifice with a 400-μm in diameter into the vacuum chamber, transported into a quadrupole ion guide, and accelerated by 10 kV after exiting the ion guide. The electrospray droplets accelerated and focused impact a solid sample. The diameter of the droplet beam for the original apparatus was approximately 3 mm in diameter, as determined by moving the sample stage. The beam focusing is mandatory for the micro area analysis. In this paper, a newly designed objective lens system which consists of Einzel objective lens, deflector and angle aperture will be presented. The beam size was measured by observing images of secondary electrons induced by EDI irradiation. The beam diameter of the ambient EDI gun could be focused to about 40 micron with 8 kV acceleration voltage and with the incident beam current of 0.5nA. The design of micro focusing EDI gun will be presented. Some preliminary data obtained for the biological application will be given.

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PMo-182
13:30 – 14:40

Dyeing Regions of Oxidative Hair Dyes in Human Hair Investigated by Nanoscale Secondary Ion Mass Spectrometry

Toru Kojima[1], Hiromi Yamada[1], Toshihiko Yamamoto[1], Miyuki Takeuchi[2], Yasuyuki Matsushita[3], Kazuhiko Fukushima[3]

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Keywords:
NanoSIMS, Hair, dye, distribution, localization,

Novel aspects:
The isotope ion mapping image of colored hair cross-section mesured by NanoSIMS revealed the important dyeing regions, which are nanometer size, for oxidative hair coloring .

Abstract:
Oxidative or permanent hair colorant, which has many kinds of color tone and brightness, is the most popular hair coloring product by people who wish to change their natural hair color and/or cover the gray hair in the world. Colorant, which contains several types of oxidative hair dyes and alkalizer, is mixed with developer containing oxidizing agent just before use and then the resulting mixture is applied to hair for dyeing. The general mechanism of oxidative dyeing is simultaneously bleaching of melanin pigment and color development from colorless oxidative dyes in hair fiber.

Human hair is a biological material having a complicated hierarchical structure. Its predominant constituents are proteins which have various amino-acid compositions. Also, it contains water, trace elements, small amounts of lipids and pigments which are nanometer sized granule.

Although the penetration and distribution of colored dye generated from oxidative dyes in hair had been investigated by an optical microscope, it was hard to observe dye localization in fine structure of hair. In order to develop more effective oxidative hair coloring product and hair care product for colored hair, it is important to understand the dye localization in fine structure of hair.

Secondary ion mass spectrometry (SIMS) , a mass spectrometric technique, is able to provide information regarding the molecular distribution on surface of the biological material. Nanoscale SIMS (NanoSIMS) is especially able to visualize the distribution of isotope ion derived from isotopically-labeled molecules with high spatial resolution and sensitivity. In this work, the localization of colored dye generated from oxidative dyes in fine structure of human hair was investigated using NanoSIMS.

Human hairs which had never been chemically treated were used. Colorant aqueous solution containing 1,4-phenylenediamine-2,3,5,6-d_4 (pPD-d_4) , a stable isotopicallylabeled oxidative hair dye, was prepared. Oxidative dyeing was performed for 60 min at 30 °C in mixed aqueous solution of colorant and developer containing hydrogen peroxide. Colored hair were embedded in epoxy resin and made a smooth surface of hair cross-section with an ultramicrotome. NanoSIMS measurements were performed with a 16 keV Cs^+ primary ion beam to detect negative secondary ions.

Deuterium ion mapping was carried out to estimate colored dye distribution and its localization. Deuterium ions were detected from whole hair cross-section and were strongly detected from particulate regions having nanometer size in the colored hair. These regions correspond to melanin granules and it is confirmed that there are more colored dye generated from oxidative dyes in melanin granules compared with other hair regions. The NanoSIMS mapping image of deuterium ion on hair cross-section revealed that melanin granules of human hair were important dyeing regions for oxidative hair coloring.

Therefore, it is considered that NanoSIMS is the effective tool for understanding the interaction of cosmetic ingredients with the fine structure of human hair.

PMo-183 Bio-imaging with Swift Heavy Ion Beams

11:10 – 12:20

Toshio Seki[1,2], Sho Shitomoto[1], Shunichiro Nakagawa[1], Takaaki Aoki[2,3], Jiro Matsuo[1,2]

[1]Kyoto University, Uji, Japan, [2]CREST,Tokyo, Japan, [3]Kyoto University, Kyoto, Japan

Keywords:
SIMS, Bio-imaging, swift heavy ion

Novel aspects:
The high secondary ion sensitivity of molecules for bio-imaging was realized with swift heavy ions.

Abstract:
The importance of imaging mass spectrometry (MS) for visualizing the spatial distribution of molecular species in biological tissues and cells is growing. SIMS imaging has been used to visualize elemental distribution at the cellular level because of its low molecular ion yield. In conventional SIMS with keV-energy ion beams, elastic collisions occur between projectiles and atoms in constituent molecules. The collisions break the molecules and produce fragments, which makes acquisition of molecular information difficult. In contrast, MeV-energy ion beams excite electrons and enhance the ionization of high-mass molecules, and a SIMS spectrum of ionized molecules can be obtained. In a previous study, we have developed a new system for imaging mass spectrometry using MeV-energy heavy ion beams, termed MeV-secondary ion mass spectrometry (MeV-SIMS) , and demonstrated more than 1000-fold increase in molecular ion yield from a peptide sample (1154Da) , compared to keV ion irradiation. In addition, we successfully obtained mass spectrometric imaging of the deprotonated peptides (m/z 1153) without any matrix enhancement [1] .

To compare MeV-SIMS with conventional SIMS, we obtained molecular imaging of rat cerebellum with MeV-SIMS and conventional SIMS with Bi_3 ions that is know as the best ions to emit secondary molecular ions in convensional probe ions. Both MeV-SIMS and conventional SIMS images of m/z 184 were clearly observed and represented well the structure of the sliced cerebellum in the optical microscope image. A fragment ion and headgroup of phosphatidylcholine were observed at m/z 184. On the other hand, it is very difficult to see the distribution of the molecule of m/z 772 in case of Bi_3 ion. This is mostly due to the low secondary ion yields and we could not get much signals with this beam. However, the molecular distribution was clearly observed with swift heavy ion beam. The high secondary ion sensitivity with swift heavy ions is very useful for biological material analysis.

[1] Y. Nakata, et al. J. Mass Spectrom. (2009) 44, 128-136

PMo-184 Secondary Ion Emission with Methanol Gas Cluster Ion Beam Irradiation

13:30 – 14:40

Shunichiro Nakagawa[1], Tosio Seki[1,4], Takaaki Aoki[3,4], Jiro Matsuo[2,4]

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Keywords:
Cluster ion, Etching

Novel aspects:
We used a methanol cluster ion to the primary beam of the SIMS.

Abstract:
When etching a substrate such as Si, the chemical etching technique is used to enhance etching. In particular, the effect of chemical reactivity under ion beam irradiation has been demonstrated when the irradiation energy was below 100 eV. With gas cluster ions bombardment, the energy is a few eV per atom, and the surface damage is much smaller than with conventional ion bombardment. Enhancement of sputtering and secondary ion emission, has been reported for organic and inorganic materials under cluster bombardment, and the effect was called "nonlinear effect ". In this study, we measured the etching depth of Si and investigated secondary ion from its surface with GCIB irradiation.

The cluster ion beam SIMS apparatus consisted of a bubbling chamber, a source chamber, an ionization chamber and an analytical chamber. Neutral gas clusters are formed by adiabatic expansion of high pressure gas through a nozzle in the source chamber. The source gas of the ion beam was Ar and a mixture of noble gas (Ar) with methanol was the reactive gas. The source gas was generated with the bubbling technique. We expected ethanol to function as a reactive gas during the irradiation. The cluster was ionized and accelerated to about 10 to 15 eV per cluster in the ionization chamber and the subsequent ionized cluster was irradiated toward the target.

As a result, the sputter depth was two to three times larger with methanol cluster irradiation than with Ar. This difference suggests that there may be a chemical etchant effect with irradiation with methanol cluster. In the SIMS spectrum with methanol cluster ion beam secondary ion emission of the molecule $(SiCH_3)_2$ was observed, suggesting that the compound was formed in reaction between the reactive gas and the substrate surface atoms under the irradiation of cluster ion beam. Methanol cluster ion beam can realize to etch to that reactively and enhance etching speed. This effect of reactive cluster irradiation is useful for high-sensitive SIMS imaging.

PMo-185 Development of Electrostatic Quadrupole Lens for MeV-SIMS Imaging

11:10 – 12:20

Sho Shitomoto[1], Toshio Seki[1,2], Takaaki Aoki[1,2], Jiro Matsuo[1,2]

[1]Kyoto University, Kyoto, Japan, [2]CREST, Tokyo, Japan

Keywords:
MeV-SIMS, Electrostatic Quadrupole Lens, Molecular Image

Novel aspects:
We succeeded in developing the focus lens for MeV-SIMS imaging and reducing measurement time.

Abstract:
Imaging mass spectrometry is a highly sensitive, chemically specific, and label-free analytical tool that provides information on the spatial distribution of molecules in biological tissues and cells. This technique is expected to be useful for pharmacokinetic visualization in vivo, various applications in drug development, and other uses.

Matrix-assisted laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS) are soft ionization techniques. MALDI uses a matrix to induce soft ionization efficiency. This probe is ultraviolet laser radiation. It has been successfully applied in imaging of large molecules of more than 10 kDa like biomolecules. However, the spatial resolution of MALDI imaging is more than several tens micrometer. Biological molecular imaging in cell needs for spatial resolution of a micrometer. Therefore, MALDI imaging is mainly used for mapping the molecules in tissue, not cell sections. SIMS analysis uses keV energy ion beams as probe and not to need matrix. SIMS provides good spatial resolution below several dozen nanometers. In general, SIMS analysis is used for imaging of elements or molecules below 500 Da, because of the low yields of high mass ions. Thus, we need a soft ionization technique for biological molecular imaging about more than 500 Da. Moreover, we require spatial resolution below a micrometer.

The technique of SIMS with MeV-energy ion beam has a great potential for providing high resolution molecular images of cells. In conventional SIMS with keV-energy ion beam, elastic collisions occur between projectiles and constituent atoms in molecules, and this phenomenon breaks the molecules and produces fragments. In contrast, an MeV-energy ion beam can excite electron and enhance the ionization of high-mass molecules, and a SIMS spectrum of ionized molecules can be obtained. However, at present, obtaining molecular imaging data takes a long time because the current density of the primary beam is quite low. To focus the swift heavy ion beam and reduce measurement time, we have developed the electrostatic quadrupole lens (Q lens) . The Q lens is doublet.

To characterize the properties of this lens, we measured the current density of the swift heavy ion passing through the lens. The primary ion beam was 6 MeV Cu^{4+} heavy ion beam. The current density increased by a factor of 33. Therefore, the measurement time can be reduced with the lens by a factor of about 30.

We could get successful secondary ion imaging of cholesterol from the cerebellum of a rat brain. The MeV-SIMS image has 4 mm ´ 4 mm field of view with a pixel size of 40 mm. A clear image was obtained and measurement time was reduced with the lens. The total measurement time was about 5 min with the lens and about 167 min without this lens. We succeeded in developing the focus lens for MeV-SIMS imaging and reducing measurement time.

PMo-186
13:30 – 14:40

Evaluation of the Damage on a Polymer Sample Caused by Shave-off Section Processing

Teruhiko Tobe, Yoshihiro Morita, Atsuko Yamazaki, Makiko Fujii, Masanori Owari

The University of Tokyo, Tokyo, Japan

Keywords:
ToF-SIMS, FIB, cross section, damage evaluation, organic materials

Novel aspects:
We proposed the novel method for low damage section processing, shave-off scanning, and evaluated the surface roughness and the amount of ion mixing.

Abstract:
The time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful tool for elemental and/or molecular imaging, because it has high spatial resolution and high detection sensitivity. Furthermore, it is possible with SIMS to put together a 3D image by stacking 2D images of each cross section fabricated with ion beams. 3D imaging of organic materials or bio-materials with ToF-SIMS has been extensively studied in recent years. However, ToF-SIMS 3D imaging has not been applied to organic materials or bio-materials practically. One of the significant problems of practical 3D imaging of these materials with SIMS is the damage caused by ion beams during etching process. In the case of sample etching with ion beams, the irradiation of beams causes knock-on-mixing effects (implantation of ions and damage to the sample structure) and the surface roughness. Especially in etching of bio-materials or organic materials, the chemical states of samples after etching differ significantly from those of unirradiated sample. To date, a great deal of efforts has been made to reduce these damage, and some effective methods have been developing. For example, the etching with cluster ion sources (SF_6^+, C_{60}^+, Ar cluster, etc.) causes less damage than that with atomic ion sources. However, they can not remove the damage and induce surface roughness completely.

We have been developing a novel etching method with Ga^+ focused ion beam (Ga^+ FIB), which is called shave-off scanning, in order to reduce the beam-induced damage. In the conventional method (the raster scanning), knock-on-mixing effects occur and damage the sample surfaces and structure seriously because of vertical or oblique beam irradiation to the sample surfaces. In the shave-off scanning, it is thought that knock-on-mixing effects are suppressed because of horizontal ion beam irradiation to the sample surface. In addition to the suppressing of knock-on-mixing effect, the shave-off scanning is superior to raster scanning in some points ; absolute depth scale, and application to rough surface and/or hetero interface. However, due to unique position between FIB and samples, the beam-induced damage on organic materials with shave-off scanning has been evaluated insufficiently.

In our previous study, molecular dynamics simulations were performed to investigate the damage to aluminum model samples with shave-off scanning. The simulations showed that shave-off scanning had higher sputtering yield and caused lower damage compared to raster scanning. On the other hand, they indicated that knock-on-mixing effects occurred to some extent even in shave-off scanning mode. Moreover, these results suggested that shave-off scanning damages organic material surface and the chemical state of its surface are not preserved after shave-off section processing.

In this study, we evaluated the damage on organic samples (6-nylon) after shave-off sectioning with Ga^+ FIB. The chemical states of 6-nylon samples sectioned with Ga^+ FIB shave-off scanning were analyzed with XPS, and the roughness of cross sections was evaluated with AFM. We investigated the effects of some parameters of sectioning (incident energy, scan speed in depth direction, etc.) on sample damage. In addition, these cross sections were analyzed by ToF-SIMS and measured the secondary ion yields of molecular ions. From these results, we discussed the optimized Ga^+ FIB shave-off processing conditions of organic materials.

PMo-187 Study on Depth Resolution Factor of Shave-off Depth Profiling

11:10 – 12:20

Makiko FUJII, Yuya HANAOKA, Tatsuya TAKETSUGU, Masanori OWARI

The University of Tokyo, Tokyo, Japan

Keywords:
SIMS, depth profiling, depth resolution, 3DAP, sputtering

Novel aspects:
We newly investigated the various factors that determine depth resolution of shave-off depth profiling by means of 3DAP and some other analytical methods.

Abstract:
In recent years, the establishment of local part analysis methods in a limited area has been aspired for a semiconductor and an electronic device industry. We have been developing shave-off depth profiling with nano-beam secondary ion mass spectrometer (nano-beam SIMS), one of the powerful methods that can be applied to the analysis of such materials and devices. Shave-off depth profiling achieves the highly precise depth profiling with nanometer-scaled depth resolution by utilizing a Focused Ion Beam (FIB) micro-machining process. We can acquire a depth profile by shave-off scan mode (A fast horizontal sweep of FIB is combined with the very slow vertical sweep). Shave-off depth profiling has its own features : absolute depth scale, pin point depth profiling and application to rough surface and/or hetero interface. In our previous studies, we obtained highly precise depth profiles of real-world samples. In addition, shave-off scan mode has been applied for low damage section processing to realize the highly accurate three-dimensional microanalysis.

The various factors that determine the depth resolution of conventional SIMS depth profiling have been investigated in detail for many years (i.e. ripple formation, mixing effect, etc.). On the other hand, the discussion of the depth resolution factor of shave-off depth profiling is still insufficient because shave-off scan mode has distinctive position of the primary ion beam against the sample. In our previous study, the amount of the mixing effects under shave-off scan mode was evaluated using Molecular Dynamics (MD) simulations. From these results, it was proved that shave-off scan mode had the high sputtering yields and the low mixing effects compared to conventional raster scan mode. In addition, the impact energy dependence on the sputtering yields and the mixing effects under shave-off condition was demonstrated. As mentioned above, there are many kinds of depth resolution factors for conventional SIMS depth profiling and the similar factors can be thought to exist for shave-off depth profiling. In this study, we experimentally investigated the depth resolution factor of shave-off depth profiling by means of various analytical tools.

Three-Dimensional Atom Probe (3DAP) has been developed for highly precise three dimensional reconstruction imaging with nearly atomic level spatial resolution. This method is effective in investigating the damage depth and the mixing effect of the sample after ion bombardment under shave-off scan mode because both 3DAP and shave-off depth profiling adopt the same sample preparation method utilizing FIB lift-out method. We prepared the sample of box shape by means of FIB micro-machining and shaved it off partly under shave-off condition. Then, the sample was processed again to needle shape and 3DAP measurement was performed to investigate the damage depth and the mixing effect caused by shave-off process. Some other analytical methods were adopted in order to evaluate the sputtered surface. The results obtained from the sample sputtered by shave-off scan, conventional raster scan, and without sputtering were compared and the amount of mixing effects was evaluated.

PMo-188
13:30 – 14:40

Tracing photosynthetic product accumulation in xylem cell wall using carbon isotope labeling

Miyuki Takeuchi, Akira Isogai

The university of Tokyo, Tokyo, Japan

Keywords:
cell wall, carbon allocation, NanoSIMS, isotope labeling

Novel aspects:
Carbon flow in a tree was traced using carbon isotope pulse labeling combined with NanoSIMS analysis. Incorporation of photosynthetic products into xylem cell wall of each cell was revealed.

Abstract:
Trees fix CO_2 in the atmosphere by photosynthesis. A part of the photosynthetic products are accumulated in tree for a long time and carbon accumulation in the tree stem is an important factor on allocation of assimilated carbon and then, ecosystem function of the tree. The main storage of carbon in trees is thick cell wall of xylem cells in stem and arrays of the cells form wood with tree rings. Environmental conditions are recorded in the tree rings during their growth[1]. For example, carbon isotopic ratio reflects environmental conditions at the time of their photosynthesis. Thus, the information in the tree rings is used for investigation of plant response to environmental factors, and it can be used as a tool in reconstructing past changes in climate. On these studies, variation of carbon isotope composition in tree rings is focused and seasonal and monthly variation pattern has been shown. Carbon transport and allocation in trees has been also studied using ^{14}C or ^{13}C labeling, though it was difficult to know time lags between photosynthesis and carbon accumulation in the stem and effects of photosynthesis on cell wall formation in a single xylem cell.

In this study, we aimed to trace transfer photosynthate that is produced in a short period, and its incorporation to xylem cells wall. Stable isotope labeling was combined with a high resolution secondary ion mass spectrometry (NanoSIMS). ^{13}C labeled CO_2 was applied to a 4-year-old *Populus euramericana* for 2 hours and the sample was harvested after 5 days. The small pieces of the xylem was fixed with 3% glutaraldehyde and embedded in epoxy resin. ^{12}C and ^{13}C analysis on the semi-thin section was performed using NanoSIMS 50L.

High resolution carbon mapping with NanoSIMS revealed ^{13}C tracer distribution within cell wall and a ^{13}C rich layer was observed in the cell wall of young xylem cells. This ^{13}C rich layer indicates that the layer was accumulated during the 5 days between $^{13}CO_2$ application and sampling. The position of the layer in the cell wall varied from innermost layer to compound middle lamella, and it reflects the developmental stage of the cells when they received ^{13}C labeled materials. These results suggest that it is possible to estimate cell wall formation process in a day or a few hours scale and it will help us to understand correlation between cell wall formation and environmental condition such as diurnal differences.

1) D. McCarroll and N. J. Loader (2004) Quaternary Science Reviews. 23, 771-801

PMo-189
11:10 – 12:20

Acceleration of gluconeogenesis of the host liver bearing human colon cancer metastases revealed by microscopic imaging mass spectrometry

Akiko Kubo[1], Mitsuyo Ohmura[1], Tsuyoshi Nakanishi[3], Shigeki Kajihara[3], Kiyoshi Ogawa[3], Makoto Suematsu[1,2]

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Keywords:
imaging mass spectrometry, metabolome, CE-MS, gluconeogenesis, tumor metastasis

Novel aspects:
Our results collected by a novel semi-quantitative IMS suggest that tumor bearing-liver utilize gluconeogenic substrates not only producing glucose to maintain its plasma level, but also stimulated glycogen storage.

Abstract:
Imaging mass spectrometry (IMS) allowed us to collect spatial information of many different metabolites in a single frozen section, and combination with capillary electrophoresis (CE) -MS data collected from the corresponding serial section provided semiquantitative information of the individual MS signals[1,2]. This study aimed to examine alterations in carbohydrate metabolism of the host liver of superimmunodeficient NOG mice that is invaded by metastases of human colon cancer xenografts in vivo ; during the development of metastases, the host mice suffer from systemic hypoglycemia.

NOG mice were intraperitoneally injected with excess amounts of $^{13}C_3$- L-alanine to determine its conversion to the mass-labeled gluconeogenic metabolites under overnight-fasted conditions. Livers under fasted conditions were collected at 0, 5 and 15 minutes and 60 minutes after the L-alanine injection. Liver tissues were removed and snap-frozen in liquid nitrogen, and 5 -micrometer cryosections were mounted on ITO-slides followed by being sprayed with 9 -aminoacridine to conduct microscopic MALDI-IMS in negative ion mode. The adjacent thick section of a frozen tissue was used for metabolome analyses by CE-MS. For semi-quantitative analysis, MALDI IMS peaks were automatically extracted from each regions of interest (ROIs) by using IMS convolution software[3]. Extracted intensity values are normalized with quantitative results by CE-MS, and used to assign region-specific values of individual metabolites [4] . The ^{13}C-labeled gluconeogenic metabolites were produced in both control and tumor-bearing livers. Greater than 2 folds of labeled *UDP-glucose* were detected in parenchyma of the tumor-bearing liver versus the control. MS^2 analyses under loading $^{13}C_3$-L-alanine provided evidence for the mass-labeled UDP-glucose with ^{13}C-containing-hexose structure in the parenchyma of tumor-bearing liver, suggesting biotransformation of L-alanine is accelerated for glycogen synthesis rather than for supporting glucose delivery to circulation. Our results collected by a novel semi-quantitative IMS suggest that tumor bearing-liver utilize gluconeogenic substrates not only producing glucose to maintain its plasma level, but also stimulated glycogen storage, leading to hypoglycemic stress under the tumor-bearing conditions. Overall, IMS combined with CE-MS analyses serves as a potentially useful strategem to carry out semi-quantitative analyses of tumor bearing liver *in vivo*.

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Acknowledgements
This work was supported by JST, ERATO, Suematsu Gas Biology Project as well as by Research and Development of the Next-Generation Integrated Simulation of Living Matter, a part of the Development and Use of the Next-Generation Supercomputer Project of MEXT (to M.S.) . Imaging mass spectrometry was supported by Grant-in-Aid for SENTAN from JST (A.K.) .

PMo-190 Early Detection of Gastric Cancer using ToF-SIMS Metabolomic Imaging

13:30 – 14:40

Yong-Chien Ling[1], Shiou-Ling Lei[1], Chun-Chao Chang[2], Chia-Lang Fang[3], Fu-Der Mai[4]

[1]Department of Chemistry, National Tsing Hua University, Hsicnhu, Taiwan, [2]Department of Internal Medicine, Taipei Medical University, Taipei, Taiwan, [3]Department of Pathology, Taipei Medical University, Taipei, Taiwan, [4]Biomedical Mass Imaging research Center, Taipei Medical University, Taiwan

Keywords:
Metabolomic imaging, ToF-SIMS, Gastric cancer；Early detection, Chemometric processing

Novel aspects:
The first attempt for early detection of gastric cancer using ToF-SIMS metabolomic imaging by ToF-SIMS spatially-resolved detecting small organic/inorganic species to yield highly specific profile followed by chemometirc grouping.

Abstract:
Metabolomics involves comprehensive, simultaneous, and systematic characterization of small endogenous metabolites in a biological system. ToF-SIMS imaging is capable of detecting organic and inorganic species distribution in gastric tissues, possessing the potential for early detection of gastric cancer which greatly increases the chances for successful treatment. Herein, we have collected four gastric tissue samples from patients pathologically classified as at stage from early to late IA (patient 1), II (patient 2), and IIIA (patient 1 and 3). A ToF-SIMS IV using a primary ion gun of 25 keV Ga^+ at typical 1 pA pulse current was used to acquired ion images from region of interest in both normal mucosa and tumor cell. Statistically low level of Fe, PC, PE and uric acid and high levels of propanoic acid, malic acid, citric acid and hexadecanoic acid were observed in positive ion ToF-SIMS results；whereas statistically low level of S and CN were observed in negative ion ToF-SIMS results. Similar metabolic profiles were prevalent among all samples. Principal component analysis (PCA) of metabolic profiles (patients 2,3,4 and 1,2,3,4, based on from PI and NI) reveals distinctive grouping between tumor and normal tissue. Additional PCA of cancer-related metabolic profile reveals distinct grouping between tumors at different stage, suggesting the potential of metabolomic imaging by ToF-SIMS as early detection tool for gastric cancer.

PMo-191
11:10 – 12:20

Separation and identification of Structural isomers of Methylpyrazole by Differential Mobility Spectrometry

Kazuhide Inoue[1], Yasutohi Kawase[1], Masazumi Yasumoto[1], Seiji Horie[1], Shigeru Yamada[2], Ryoma Yamamoto[2]

[1]TAKARA BIO INC. Shiga, Japan, [2]K.K. AB SCIEX. Tokyo, Japan

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Keywords:
Differential mobility separation, Methylpyrazole

Novel aspects:
Differential mobility separation can separate Structural isomers.

Abstract:

[INTRODUCTION]

A lot of compounds are very similar in structure and may have identical chemical composition, making it difficult to separate these species even by accurate-mass measurements. The analysis of Methylpyrazoles is just such an application, too.

Differential mobility separation (DMS) can separate species based on the difference mobility between their high field and low field. At a specific separation voltage (SV), it is possible to ensure transmission of particular species through the DMS device to detector (MS/MS detection in this work) when a compensation voltage (CoV) is applied, while other species will simply be lost to the electrode walls. AB SCIEX SelexION™ device is technology that is using such a differential mobility separation.

In this work, an orthogonal separation technique has been utilized to differentiate between isobaric Methylpyrazoles (3-Methylpyrazole and 4-Methylpyrazole), using differential Mobility Spectrometry prior to analysis by MS/MS.

[MATERIALS and METHODS]

HPLC Conditions :

For the analysis was used at a Shimadzu XR LC system. The method was developed using a conventional ODS column with an isocratic solution of acetonitrile water containing 0.1% formic acid.

MS/MS Conditions :

An AB Sciex QTRAP (R) 5500 system with Turbo V (R) source and the electrospray ionization (ESI) probe was used. The source was operated at 550 degrees with Gas 1 and Gas 2 at 60 and 80 psi, respectively. The ion source region of the mass spectrometer was modified for incorporation of the AB SCIEX SelexION™ ion mobility separation device.

[RESULTS]

3-Methylpyrazole and 4-Methylpyrazole share the same precursor ion mass, and the same MRM transitions, the separation cannot be performed by tandem mass spectrometry alone. Traditionally the separation is accomplished using long chromatographic gradients, which is effective but which results in a reduction in sample throughput. Using the completely orthogonal separation provided by AB SCIEX SelexION™ technology, the 3-Methylpyrazole and 4-Methylpyrazole have been completely separated prior to analysis by MS/MS.

In addition it has been shown that the AB SCIEX SelexION™ technology can significantly reduce LC-MS/MS run-times, since complete chromatographic separation of potentially interfering species is no longer required.

PMo-192 Investigation of an IMS-TOF MS with a laser based ionisation under atmospheric conditions

13:30 – 14:40

Marvin Ihlenborg, Tassilo Muskat, Juergen Grotemeyer

CAU Kiel, Kiel, Germany

Keywords:
IMS, MPI

Novel aspects:
Comparison of the ion yields obtained by a MPI and ionisation by an tritium source.

Abstract:

Introduction

In the past an Ion Mobility Spectrometer (IMS) was purchased from *Draegerwerk AG* by our group. Under atmospheric pressure conditions this type of IMS uses a ^{3}H-source to produce ions. To extant the range of analytes, which are not easy to protonate, the^{3}H-source should be replaced by a laser based ionisation method. Additionally other types of ions are formed (e.g. radical cations instead of protonated species) and due to the wavelength of the laser the ionisation is selective. Furthermore the existing apparatus (cf. method) should mainly remain the same.

Method

The mainly used apparatus is described earlier [1] only a short overview will be given here. A home build and a purchased IMS (*Draegerwerk AG*) are coupled to a home build time of flight mass spectrometer (TOF-MS) . To transfer the ions from the atmospheric pressure IMS to the high vacuum system of the TOF-MS several different vacuum stages have been incorporated.
In addition a collecting lens is build into the existing setup. Due to this changes the potentials of the different transfer units are aligned to maintain the ion transfer the same.

Preliminary Data

In earlier experiments [1] it can be seen that during the transfer of the ions from the high pressure system to the high vacuum stage of the TOF-MS a high loss of ions occurs. To compare the different ionisation methods first measurements were made with the ^{3}H-source and acetone respectively toluene as analytes. The transferred ions through the IMS were observed with a Faraday Cup connected to an analogue amplifier. Afterwards the ^{3}H-source was displaced by a laser beam focused directly in front of the entrance of the IMS. The laser beam was obtained by the 4th harmonic of a Nd : YAG laser. Furthermore a repeller plate was incorporated in front of the IMS. The measurements with toluene were repeated due to the good ionisation capability by 266 nm by a multi photon ionisation process (MPI) .

Several effects can be observed by changing the ionisation technique. The obtained signal rises in intensity by the tenfold in addition to a broadening with a distinctive tailing. Furthermore the signal maximum is shifted by approximately 2,5 ms to later drift times.

PMo-193 Separation of Isobaric Phosphopeptides using Differential Ion Mobility Spectrometry with Tandem Mass Spectrometry.

11:10 – 12:20

Chie Inagaki, Masato Aoshima, Takeshi Shibata, Shigeru Yamada, Sumie Ando

KK ABSCIEX, Tokyo , Japan

Keywords:
Ion Mobility, Phosphopeptide, MRM

Novel aspects:
DMS is able to separate and quantitate identical phoshopeptides which have a different phosphorylation site. It helps to understand cell signaling pathway.

Abstract:

Introduction

Protein phosphorylation is a key regulator of cellular signaling pathways. This highly controlled biochemical process is involved in most cellular functions and defects in protein phosphorylation has been implicated with many diseases including cancer, highlighting the importance of understanding phosphorylation-mediated signaling networks.

Much work has been done over the last few years to develop Multiple Reaction Monitoring (MRM) based strategies to enable and accelerate the transition from discovery to targeted quantitative MS verification assays because they are not necessarily dependent on the use of antibodies and can therefore be rapidly and cost-efficiently developed in comparison to traditional enzyme linked immune sorbent assays (ELISA) . However, it is difficult to identify and resolve phosphopeptides which have identical amino acid sequences and differ only in the site of phosphorylation even if using ELISA and MRM.

We report here to use a QTRAP 5500 System equipped with a differential mobility separation device for identification and separation of identical phoshopeptides which have a different phosphorylation site.

Material and Methods

In this work, we used MS PhosphoMix standards (SIGMA-ALDRICH Corporation) containing phosphopeptides, ADEPpSSEESDLEIDK (M1) and ADEPSSEEpSDLEIDK (M2) derived from HeLa cells. A 5500 QTRAP system with Turbo V source and the electrospray ionization (ESI) probe was used. MRM transitions for M1 and M2 of phosphopeptides were developed and used to measure the calibration curves. For the differential mobility separation experiments, a QTRAP 5500 LC/MS/MS system equipped with SelexION Technology (a differential mobility separation device) was used. Isopropanol was used as a chemical modifier and was introduced into the curtain gas using an integrated pump.

Results

The SelexION Technology is a planar differential mobility separation (DMS) device that attaches between the curtain plate andorifice plate of the 5500 QTRAP system. DMS can separate ions in the gas phase based on their different migration rates in high and low electric fields and is orthogonal to both MS and liquid chromatography.

Even if we could detect both M1 and M2 phosphopeptides from MS PhosphoMix Standards using MRM mode without DMS, they were not resolved chromatographically. When using isopropanol as a chemical modifier in the DMS cell, M1 and M2 exhibit different mobility coefficients and were completely separated by using different compensation voltages (CoV) with an optimized separation voltage (SV) . DMS provided the capability to greatly separate the phosphopeptides where phosphorylation was different location.

Although both M1 and M2 phosphopeptides were not resolved chromatographically in this case, their MRM transitions were identical and their separation using DMS was essential to their quantitation. Furthermore, we performed the calibration curve and statistics in triplicate for M1 and M2 in MRM mode with DMS. It was good linearity that correlation coefficient (r) >=0.99 in concentration range 10-1000 fmol/μL on column and accuracy was between 90% and 110%. The calibration curve was performed without any internal standard and %CV was within 5 %.

Conclusion

We suggest that SelexION differential ion mobility technology is dramatically able to separate and quantitate localization variants of phosphopeptides to understand the dynamic changes of the proteome, the crosstalk and selectivity of different phosphorylation, and their impacts on signaling cascades.

PMo-194
13:30 – 14:40

Integrative structure determination of protein assemblies using mass spectrometry, ion mobility and cross-linking.

Argyris Politis[1], Zoe Hall[1], Florian Stengel[2], Ruedi Abersold[2], Carol V Robinson[1]

[1]University of Oxford, [2]ETH Zurich

Keywords:
IM-MS, CXMS, Integrative structural biology

Novel aspects:
Integrating, for first time, ion mobility-mass spectrometry and cross linking technology for structural determination of macromolecular assemblies can make a significant contribution to hybrid structural biology.

Abstract:
Recent advances in the field of mass spectrometry (MS) and proteomics have added a whole new dimension to the study of protein-protein interactions and the architecture of protein complexes. The additional coupling of ion mobility (IM) spectrometry with MS has enabled the two-dimensional separation of gas-phase ions according to 'size' and mass-to-charge ratio. Here, we describe an integrative approach to structure characterisation of macromolecular protein assemblies. To this end, we combine native MS of intact complexes and subcomplexes, IM and cross-linking technology (CXMS) . Subcomplexes are obtained from solution-phase disruption experiments in which chaotropic agents or manipulation of ionic strength is used to perturb protein interfaces. Stable subcomplexes, subunit packing, and pair-wise interactions can be used to reveal the building blocks of protein complexes. Specifically, MS of the intact complex, with its subcomplexes, can provide information on the stoichiometries and connectivities of subunits within heterogeneous assemblies. Both CXMS and IM provide information on topology. Whilst CXMS reveals data on the inter- and intra-protein proximity, information on the overall topology of a protein complex can be attained by the collision cross section (CCS) from IM. These experiments can be used as spatial restraints in modelling the structures of protein assemblies which are difficult to study by conventional structural biology approaches.

Here, we explore in detail the strengths and limitations of using IM-MS for structure determination by disrupting six heteromeric complexes in solution. Experimental CCS of protein complexes and their subcomplexes agreed well with those calculated from their crystal structures. This indicates minimum perturbation of tertiary structure following the disruption of subunit-subunit interactions in solution. Next, we benchmarked our modeling approach on several known protein complexes, with different topologies and complexities, using experimental data from IM-MS. Candidate models for heteromeric complexes tryptophan synthase (143 kDa, 4-mer) and nitrobenzene dioxygenase (218 kDa, 6-mer) were evaluated by incorporating a scoring function which measures their closeness-of-fit to the experimental data. The best-scored model for these complexes showed good structural similarity to the native structure, with centroid RMSD < 9 Angstroms. Finally, we applied our integrative approach on a protein assembly of high biological interest, the proteasome lid from *Saccharomyces cerevisiae*, for which no high resolution structure exists. This was achieved by combining information on connectivity, subcomplex topology and protein-protein interactions from CXMS and IM-MS. The best-scored model structure was found to be in excellent agreement with recently published electron microscopy data of the lid (Lander et al., Nature, 2012) , clearly revealing the synergistic effect of combining multiple techniques.

Overall we show that IM-MS can play a useful role in structural characterisation of assemblies, particularly when integrated with information from other sources, such as CXMS. Our MS-based approach can therefore make an important contribution to hybrid structural biology and integrative modelling approaches, providing insights into both the structure and function of macromolecular machines.

PMo-195
11:10 – 12:20

Advances in Chip-based FAIMS/Triple Quadrupole MS: Instrumental Studies and Analytical Capabilities

Richard A Yost[1], Christopher R Beekman[1], Chia-Wei Tsai[1], Michael Ugarov[2], George Stafford[2]

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Keywords:
FAIMS, DMS, ion mobility separation, tandem mass spectrometry, triple quadrupole

Novel aspects:
The first demonstration of interfacing a micro-FAIMS cell to a triple quadrupole mass spectrometer for fast-scanning FAIMS.

Abstract:
High-field asymmetric waveform ion mobility spectrometry (FAIMS) separates ions based on differences in ion mobility at high and low fields, and offers a novel approach to component separation in mass spectrometry. A variety of FAIMS electrode geometries are available, including planar and curved geometries. A micromachined FAIMS chip, an order of magnitude smaller than conventional FAIMS electrodes, offers a number of advantages for FAIMS/MS. The chip is composed of multiple micro-channels with a gap of <100µm ; therefore, the field strength required for separation is achieved with lower voltages compared to conventional FAIMS devices. The small size of the FAIMS chip and low operational voltages enable more rapid scanning of dispersion fields (DF) and compensation fields (CF) than with conventional FAIMS cells.

Data were collected with an Agilent 6460 triple quadrupole mass spectrometer (TQMS) . A custom Owlstone FAIMS chip (micro-FAIMS) with a gap width of 100µm and thickness of 700µm was mounted in front of the sampling capillary. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were employed as ionization sources. The FAIMS waveform was generated with a custom Owlstone waveform generator providing a DF range of 0 to 350 Townsends (Td) and a CF range of -30 to 30Td (equivalent to a DV range of 0 to ~600V and a CV range of ~-50 to +50V) .

The fast scan ability of the microscale FAIMS device offers unique capabilities. For instance, a complete CF/DF scan can be performed in a matter of seconds, permitting optimization of DF and CF for individual mixture components. Complete 3-dimensional CF/DF plots for selected ions or selected MS/MS reactions can identify and differentiate co-eluting isobaric and isomeric compounds. Fast CF/DF scanning permits dynamic programming of FAIMS and MS/MS settings, including rapidly jumping between selected points in CF/DF space to separate individual mixture components.

For example, the three isomeric phthalate ions (ortho-, meta-, and para-) were analyzed by ESI/FAIMS/MS in full-scan MS mode and in selected ion monitoring (SIM) mode to characterize scan speed, FAIMS resolution, and transmission. Tandem mass spectrometry experiments (ESI/FAIMS/MS/MS) were also performed in selected reaction monitoring (SRM) mode. The DF was scanned from 50 to 350Td (DV of ~85 to 600V) with up to 66 steps of ~4.5Td each, and the CF was scanned with up to 150 steps from -3 to +7 Td (CV range of about 17V) to generate 3-dimensional CF/DF/intensity plots) . The use of SIM or SRM mode and a smaller number of CV and DV steps dramatically shortens the scan time, providing complete CF/DF scans 10-100x more rapidly than with a conventional FAIMS cell.

In other experiments, the effect of the addition of carrier-gas modifiers on FAIMS separation has been explored. Adding carbon dioxide, for instance, dramatically shifts CF values, significantly improving the separation of the isomeric phthalate ions, as well as separation of dimeric and trimeric ions.

This presentation will report the first experiments interfacing a micro-FAIMS cell to a triple quadrupole mass spectrometer for fast-scanning FAIMS.

PMo-196
13:30 – 14:40

Selected Mobility Accumulation (SMA) in a Trapped Ion Mobility Mass Spectrometer (TIMS-MS)

Ulrich Giessmann, Melvin A Park, Desmond Kaplan, Mark Ridgeway

Bruker Daltonics, Billerica, MA, USA

Keywords:
Trapped Ion Mobility Spectrometry, Selected Mobility Accumulation, Comparison with conventional IMS, IMS for TOF analyzers, IMS for FTMS analyzers

Novel aspects:
A new method of selected mobility accumulation (SMA) of ions in a trapped ion mobility spectrometry (TIMS) analyzer.

Abstract:
Introduction:
Recently, a new type of ion mobility analyzer the "Trapped Ion Mobility Spectrometry " (TIMS) analyzer was introduced, which produces results similar to conventional drift cell analyzers in a physically smaller design (~10X) and at much lower operating potentials [1, 2] . However, an analysis via TIMS as described thus far proceeds too rapidly (~ 2 ms/peak) for any mass analyzer except time-of-flight (TOF) to follow. In the present a new technique - selected mobility accumulation (SMA) is introduced, which matches the timescale of the mobility analysis with that of "low duty cycle " mass analyzers (e.g. FTMS) and techniques (e.g. ETD) . SMA is achieved by efficiently accumulating ions of the desired mobility for long (300 ms) periods of time.

Results:
In the present work, a TIMS analyzer is incorporated as a section of an ion funnel in the ion source of a mass spectrometer. As described previously [1,2] , in "broadband " mode, ions are initially trapped in the TIMS analyzer wherein gas flow pushing ions downstream is counterbalanced by an electric field holding the ions back. After trapping, the electric field is slowly decreased so that ions are eluted according to mobility. Eluting ions are mass analyzed in the orthogonal time-of-flight analyzer to produce a TIMS MS spectrum. To perform selected mobility accumulation (SMA) experiments, the electric field is modified to form a "plateau " within the TIMS trap on which ions of a selected mobility or mobility range can be accumulated.

The mobility analysis in a TIMS device may be viewed as roughly the same as that in a conventional IMS drift cell except that, in the conventional cell an electric field pushes ions through a stationary gas whereas in the TIMS analyzer a retarding electric field holds ion in place against a moving gas. Ions, initially trapped in the TIMS analyzer, elute according to mobility lowest mobility first as the strength of the retarding electric field is reduced. The "shape " of the retarding electric field, potential vs. axial position, has a profound effect on the number and types of ions that are initially trapped and the timing of their elution.
The effects of various retarding field gradient profiles were explored, and a plateau in the gradient profile at about 4 % below the highest retarding gradient resulted in the optimal SMA results, i.e. mobility selection resolution of greater than 50 and duty cycle > 50%. In one series of tests *m/z* 1222 ions from Agilent tunemix (PN G1969-85000) , which have a a Ko of about 0.72, were able to be selectively accumulated for 300 ms without signs of saturation. Ejection of these ions required less than 100 ms, thus the fundamental limit on the duty cycle is much better than 50%. In further tests, higher amplitude RF confining fields resulted in longer accumulation times before the onset of saturation. Also, higher pressures at the entrance to the TIMS analyzer leads to higher resolution SMA but lower accumulated ion abundances.

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PMo-197
11:10 – 12:20

Evaluation of the performance of microscale FAIMS for enhancement of quantitative analysis of metabolites and peptides

Robert W Smith[2], Lauren Brown[1], Danielle E Toutoungi[1], James Reynolds[2], Ashley Sage[3], Anthony W Bristow[4], Andrew Ray[4], Daniel Weston[5], Ian Wilson[5], Billy Boyle[1], Colin Creaser[2]

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Keywords:
FAIMS, mobility, quantitatation, metabolites, peptides

Novel aspects:
Use of FAIMS pre-selection with LC-MS plus in-source CID for rapid quantitative analysis of metabolites and peptides.

Abstract:
High-field asymmetric waveform ion mobility spectrometry (FAIMS) is an atmospheric pressure technique that separates ions by their differential mobility under high and low electric fields. In microscale FAIMS, the ions travel through channels with sub-millimetre dimensions, which enables substantially higher electric fields and faster separation than macroscale differential mobility devices. Separation on sub-second timescales becomes possible, allowing the device to be used during real-time LC-MS analysis. The extra separation dimension can be used to improve signal-to-noise in real-time quantitative analysis. This study evaluated the performance of a microscale FAIMS system in various analyses including the determination of urinary drug metabolites and the identification and quantification of peptides in plasma.

Microscale FAIMS devices (Owlstone Ltd, Cambridge, UK) were interfaced to an Agilent 6230 series TOF MS with a Jet Stream ESI source and an Agilent 1200 series LC. The ion channels in these devices consist of an array of parallel electrodes with either 35 μm or 100 μm gaps, to which an asymmetric dispersion field (DF) is applied. Ions with a selected differential ion mobility are transmitted through the device by application of a suitable compensation field (CF) while other ions are eliminated in the filter. At this scale, DFs of up to 300Td can be applied, and with a typical TOF MS capillary inlet gas flow rate of 1.7L/min, ion residence times are reduced to 50-250 μs. In some cases, in-source collision-induced dissociation of the FAIMS pre-selected ions (LC-FISCID-MS) was also carried out prior to mass analysis.

For the LC-FAIMS-MS analysis of metabolites in urine, (R/S) -ibuprofen 1-b-O acyl glucoronide (IAG) metabolite was spiked into filtered, diluted (x 2) urine. LC separation of the IAG spiked-urine was carried out on C18 column (Agilent Zorbax Rapid Resolution HT, 2.1 x 50mm, 1.8 μm) using an isocratic 0.2ml/min flow to achieve rapid elution of the metabolite (< 2 mins) .The FAIMS device was set at a fixed DF and CF (CF = 1.9Td, DF = 260Td) that had been previously determined from a direct infusion of a mixture of ibuprofen and IAG to give good transmission of the metabolite but not the parent drug. With FAIMS pre-selection, the spiked standards gave a linear response over two orders of magnitude with an R^2 value of > 0.99, showing an improvement in LOQ of a factor of 2 over the same experiment without FAIMS pre-selection. This was due to a doubling of the signal-to-noise ratio for the metabolite peak with FAIMS pre-selection.

The potential of a quantitative LC-FISCID-MS method was also evaluated. In this technique, the FAIMS device is used to pre-select certain peptides, which are then fragmented by setting the TOF fragmentor voltage to produce in-source CID. Aliquots of human plasma tryptic digest were spiked with the exogenous peptide gramicidin S. A DF of 275Td and CF of 3.0Td were applied to pre-select the gramicidin precursor ion. Calibration graphs for the LC-FAIMS-MS analysis of the precursor ion (m/z 571) and the LC-FISCID-MS analysis of the four FAIMS-selected in-source CID product ions (*m/z* 311,424, 685 and 798) show a linear response (R^2> 0.99) in the range 0.45-9.0 μg/mL. The %RSD for the peak area of the FAIMS-selected precursor ion (*m/z* 571) without in-source fragmentation was 5.1% (n= 6 ; 0.45 μg/mL) . LC-FISCID-MS precision for the fragment ions at *m/z* 311, 424, 685 and 798, was 13.3%, 14.6%, 13.1% and 8.2% respectively, demonstrating good reproducibility for quantitative measurement at this concentration.

The results demonstrate that microscale FAIMS is a quantitative technique applicable to both small and large molecules that can improve level of quantitation, with good precision and linearity, and that the LC-FISCID-MS method could be an alternative to LC-MS/MS for quantitative analysis.

PMo-198 Develpment of Ion Trap Mobility Measurement System

13:30 – 14:40

Toshiki Sugai, Yoshihiko Sawanishi, Masashi Shinozaki, Sho Osaka

Toho University, Funabashi, Japan

Keywords:
Ion Mobility, Ion Trap, Long term measurement

Novel aspects:
Long term structural measrements have been performed by the newly developed ion trap mobility measurement system.

Abstract:
Ion mobility/mass measurements have been utilized for various bio molecules and nano materials [1,2] . The high sensitivity and high throughputs for mixed and unstable materials have advantages to clarify the new structures and the structural changes. However, the measurement requires high pressure buffer gas which leads to ion dilution and other experimental difficulties. One of the approaches to overcome this problem is TWIMS system developed by Waters Co. Ltd. [3] It utilizes ion traps to reduce the diffusion and to enhance detection sensitivity. However there is potential demand for higher resolution and longer-term trap. To improve these properties we have developed an ion trap mobility measurement system for charged particles in the first stage.
The system consists of stacked ring electrodes and an RF and LF power supplies. The RF and LF power supplies produced the radio frequency field to trap the charged particles and the low frequency field to move the particles in the trap for the mobility measurements, respectively. The charged particles of salt water solution and polystyrene were introduced in the trap and were detected and monitored through their movement by a microscope with a laser irradiation.
The particles were trapped for more than 2 hours where their mobility of the particles was measured by the amplitude of the modulation. The amplitude of the salt solution particles decreased gradually showing the particles growth through water vapor absorption from air. On the other hand, the amplitude of the polystyrene particles was constant showing steady structures. Those results show the potential high structural resolution and high traceability of the system.
We are now developing a multi-stage ion trap mobility system with an interface for a mass spectrometer in the second stage which enables us to realize much higher resolution and application to nano materials.

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PMo-199
11:10 – 12:20

Achieving High Resolution in a Benchtop Resistive Glass Drift Tube Ion Mobility Spectrometer

WILLIAM C NETOLICKY[1], PAULA HOLMES[1], FACUNDO FERNANDEZ[2], JOE TICE[3], BRIAN MUSSELMAN[3]

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Keywords:
ION MOBILITY SPECTROMETRY, RESISTIVE GLASS, DART

Novel aspects:
An ion mobility spectrometer with very high resolving power was designed and constructed using resistive glass tubes, a photo-etched Bradbury-Nielsen gate and a thermally assisted DART ionization source.

Abstract:
Ion mobility spectrometry has become widely accepted for the detection of chemical warfare agents, explosives and narcotics as well as for pharmaceutical quality control and pesticide screening of food. However, to minimize the frequency of false positive and false negative results, it is necessary to design and construct an ion mobility spectrometer with high separation power without compromising instrument simplicity, serviceability, and cost. Resistive glass drift tubes provide key benefits such as uniform electric fields with minimal radial inhomogeneities and ease of fabrication. In addition, the single-piece construction allows even counter flow of drift gas without the need for additional containment. Reaction and drift regions of the ion mobility spectrometer were built using resistive glass technology. The Bradbury-Nielsen-type ion gate was produced using photo-etched grids and was evaluated for its performance at varying operational voltages and frequencies. Ionization sources included a corona discharge as well as a novel thermally assisted Direct Analysis in Real Time (TA-DART) source. The DART system provides a non-radioactive means for rapid desorption and ionization of both solid and liquid analytes with minimal sample preparation. Identification of volatile and solid samples was achieved with great simplicity and excellent sensitivity. Analytical performance was assessed using several different calibrants and pharmaceutical samples. Resolving power of 70-120 was measured from the mobility spectra demonstrating the ultra high resolution of the resistive glass drift tube IMS. Reduced mobility values were calculated in order to confirm the identification of the observed signals with excellent agreement to literature values.

PMo-200
13:30 – 14:40

Direct detection of S-nitrosylated peptides with UV-MALDI MS using porphyrin and retinoic acid as a matrix additive

Makoto Watanabe[1], Rie Yamamoto[1], Shinichi Iwamoto[1], Yuko Fukuyama[1], Ritsuko Tanimura[1], Shin-Ichirou Kawabata[1], Taka-Aki Sato[1], Shunsuke Izumi[2], Koichi Tanaka[1]

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Keywords:
S-nitrosylation, post-translational modification, UV-MALDI, tetraphenylporphyrin, retinoic acid

Novel aspects:
The use of tetraphenylporphyrin (TPP) or retinoic acid (RA) as a matrix additive allows the direct detection of S-nitrosylation by UV-MALDI MS.

Abstract:

Introduction:
Nitric oxide (NO) exerts a ubiquitous influence on cellular signaling, largely through S-nitrosylation/denitrosylation of cysteines in proteins. Therefore, detailed analysis of protein S-nitrosylation is important for a better understanding the action of NO in various biological processes. However, S-nitrosylation has never been detected by UV-MALDI MS because of S-NO bond instability.

Methods:
In this study, tetraphenylporphyrin (TPP) and retinoic acid (RA) were used as matrix additives with 2, 5-dihydroxybenzoic acid (DHB) to evaluate their suppressive effect on NO group dissociation of S-nitrosylated peptides. The two S-nitrosylated peptides (VFDARDC (NO) RSAQ and EMFTYIC (NO) NHIK) were subjected to MS and MS/MS analysis by MALDI-TOF MS and MALDI-QIT-TOF MS, respectively.

Results:
In MS analysis, the predicted intact molecular ions [M+H] + were observed using TPP or RA as a matrix additive although the corresponding NO-dissociated ions [M-NO+H] + were mainly observed. However, these ions [M+H] + were not observed in the absence of TPP or RA. Furthermore, MS/MS analysis of these ions indicated that the peptides contained S-nitrosylated cysteines.

Conclusion:
We demonstrated for the first time the possibility that addition of tetraphenylporphyrin (TPP) or retinoic acid (RA) to MALDI matrix solutions allows the direct detection of S-nitrosylated peptides by UV-MALDI MS. In addition, MS/MS analysis of S-nitrosylated peptides enables the modification sites to be determined.

PMo-201
11:10 – 12:20

Bactericidal effects of atmospheric ions formed in ambient corona discharges

Rena Gonda, Yuya Yamakawa, Kanako Sekimoto, Mitsuo Takayama

Yokohama City University, Yokohama, Japan

Keywords:
corona discharge, negative ions

Novel aspects:
An ambient corona discharge system which makes it possible to separately form various ionic and neutral species was established, and ionic and neutral species having bactericidal effects were specified.

Abstract:
Atmospheric ions formed in ambient electrical discharges have been applied to various research fields, e.g., the application of their sterilization effects in plasma medical sciences, as well as the utilization as reagent ions in APCI-like ambient ionization in modern mass spectrometry. However, it is difficult to regularly produce atmospheric ions and by-product neutral species in discharge area, and therefore, the effects of ionic and/or neutral species on the sterilization have not been specified by now. Recently we have identified the atmospheric ions and neutral species formed in ambient positive and negative corona discharges, and found the factors governing the formation of those chemical species in discharge area. Here we attempted to establish an ambient corona discharge system which makes it possible to separately irradiate various ionic and neutral species to *Esherichia coli* (*E. coli*) and investigate bactericidal effects of individual chemical species.

Discharge experiments were performed in the laboratory ambient air under atmospheric pressure at 25 ° C. A highly-curved electrode used was a needle with 1 μm in the tip curvature radius. An orifice plate and brass plate were utilized as opposite electrodes to detect ionic and neutral species formed in discharge area using a JMS-LCmate double-focusing MS (JEOL, Tokyo, Japan) and AXIMA-CFR time-of-flight MS (Shimadzu, Kyoto, Japan) , while in the case of the investigation of bactericidal effects, agar mediums spread *E. coli* DH 5 α suspensions of 200 μL was employed. The discharge conditions used were of 3 mm in point-to-plane electrode gap, +4.5 and -3.5 kV in DC needle voltage and 0 to 45° in needle angle with respect to the orifice axis.

The formation of atmospheric negative ions and neutral species in negative corona discharges was attributed to the electric field strength on the needle tip surface, which is determined by the electrode gap, needle voltage and angle. The ionic and neutral species formed at the tip peripheral region with the low field strength below 10^7 Vm^{-1} was hydroxide ion HO^- and carbon clusters C_n. The high field strength above 10^8 Vm^{-1} at the tip apex region led to the formation of abundant nitrogen oxide ions NO_x^- (x = 2,3) and neutral species NO_x. In the case of positive corona, oxonium ion H_3O^+ was generated under any electric field strengths, while neutral species NO_x occurred at the tip apex region with high field strength. The relationship between the field strength and the resulting ionic and neutral species can be interpreted from the standpoint of the field-emitted electron kinetic energy which is the principal factor governing the sequential progress of successive ion-molecule reactions in the formation of individual chemical species. The trajectories of the resulting chemical species in discharge area were regulated by the point-to-plane electrode configuration used and electric field distribution established. That is, the ions HO^-, NO_x^-, and H_3O^+ can move along field lines that arise from the needle tip surface where individual ions are formed, whereas the motion of the neutral species NO_x directs toward the needle tip axis due to elastic collisions with ions.

By using the discharge system described above, individual ionic and neutral species NO_x^-, HO^-, H_3O^+, C_n and NO_x were separately irradiated to sample agar mediums with *E. coli*. When the discharged agar mediums were incubated at 37° C over night, growth of *E. coli* was observed at the agar medium area where H_3O^+ and C_n were irradiated, while *E. coli* irradiated NO_x^- and HO^- ions and neutral NO_x did not grow. These results suggest that the specific chemical species, i.e., NO_x^-, HO^- and NO_x have bactericidal effects.

PMo-202
13:30 – 14:40

Thermal Activation of Methane: Why [Ti,O,H]+ and [V,O,H]+ are More Than the Sum of Their Atoms.

Robert Kretschmer, Maria Schlangen, Helmut Schwarz

Technical University Berlin

Keywords:
Ligand effects, methane activation, Ion-molecule reactions, vanadium, titanium

Novel aspects:
Discovery of intrinsic properties of transition-metal complexes and ligand effects with respect to methane activaton in the gas phase

Abstract:
Transition metal catalyzed C-H bond-activation processes constitute an active area of research which can increase the power of catalytic organometallic chemistry. Especially the activation and functionalization of methane has been identified as one of the key challenges to addressing the global energy problem.While industrial process under environmentally benign and economically feasible conditions have to be developed, the particular role of the electronic configuration of transition-metal complexes in the elementary steps of such processes have been studied in quite some length. Gas-phase studies provide an ideal arena to uncover mechanistic aspects in an unperturbed environment at a strictly molecular level. While “bare “ ground-state transition metals of the first row do not bring about C-H bond activation of CH_4,[1] ligated nickel II species $[NiD]^+$,[2] $[NiH]^+$,[3] and $[NiF]^+$[4] form the corresponding $[Ni(CH_3)]^+$ complexes. In addition, bis-ligated cations are also capable to activate methane under thermal conditions, i.e. $[CrO_2]^+$,[5] $[FeO(OH)]^+$,[6] and $[NiH(OH)]^+$.[7]
Here we present the reactions of $[M(OH)]^+$ and $[HMO]^+$ (M = Ti, V) with methane under thermal conditions. While $[Ti(OH)]^+$ does not bring about C-H bond activation, the $[HTiO]^+/CH_4$ couple forms $[TiO(CH_3)]^+$ and H_2; the same holds true for the isomers $[V(OH)]^+$ and $[HVO]^+$. These results serve as examples of both the prominent role of the oxidation state and the ligand effects.

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PMo-203

11:10 – 12:20

Measurements of Proton and Alkali Ion Affinities using CID Spectra of High Performance Tandem Mass Spectrometer

KIMIO ISA[1], SHIGEKI MATSUMURA[2], RYUJI NAKADA[3]

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Keywords:
CID, Proton affinity, Alkali Ion affinity, kinetic method, sector tandem

Novel aspects:
700T BEBE tandem mass spectrometer was high performance, especially ion intensity relatively strong and mass resolution was also good and affinity determination was easily done.

Abstract:

1990 years day, the measurements of CID spectra of a protonated dimer ion, $(M_1+H+M_2)^+$ were determined the proton affinity values or proton affinity (PA) order of amino acids M1 and M2 [1](20 amino acids) using kinetic method. JEOL 300 double-focusing mass spectrometer (EBE type with cylindrical electrostatic analyzer: one sector) was used. The following conditions were adapted: mass resolution 1000; FAB ion source, FAB gun was argon, and accelerating voltage of fast atom beam 3 kV; and CID gas argon at a pressure at $2*10^{-6}$Torr at the Penning gauge (to attenuate the intensity of the gas pressure did not have a significant effect on the CID spectra.) Hetero dimer ions was accerelated at 3 kV.

1996 years day, the proton affinity order of H_2SO_4 and 14 solvent molecules (acetone, methyl alcohol, ethyl alcohol, 1-propyl alcohol, 2-propyl alcohol, 1-butyl alcohol, 2-butyl alcohol, t-butyl alcohol, 1-pentyl alcohol, 1-hexyl alcohol, ethylene glycol, acetic acid, dimethyl sulfoxide, and water) were measured and CID spectra of the hetero dimer cluster ions were also measured. The order of proton of H_2SO_4 was also confirmed to be $PA(H_2SO_4)=707kJ/mol$[2] .

2003 years day, measurements of proton and alkali ion affinities using CID spectra of high performance tandem mass spectrometer JEOL 700T (BEBE geometry: two sectors tandem) operating under FAB ionization conditions (Xe beam of 6 keV translational energy, gun current 3 mA). Ion accelerating voltage was 10 kV. The amine cluster ion including HCl, was measured and the proton affinity of methamphetamine was determined to be 965 kJ/mol using the hetero dimer ions $(A_1+HCl+H+A_2)^+$ type ions including HCl, r value is 0.96.[3]

2009 years day, dicision of proton affinity of amino acids and dipeptide including glycine using kinetic method[4] by FABMS and FABMS/MS. And the behavior of two types samples are compared. The r values are almost 0.99. The measured samples are 9 amino acids (Cys, Ser, Asp, Val, Thr, Leu, Ile, Phe, and Met) and 12 dipeptides (Gly-Ser, Gly-Val, Gly-Leu, Gly-Asp, Gly-Thr, Gly-Ile, Gly-Phe, Gly-Met, Ser-Gly, Thr-Gly, Phe-Gly, and Ala-Gly). The comparison and the examination of these PAs are done.

2010 years day, alkali metal ion affinity of glycerol was determined by means of MS/MS. The experimental results were analyzed by kinetic method (Cooks et al. proposed 1978). Both Na^+ and Li^+ ion affinitys of glycerol were determined to be 181.8(kJ/mol) and 261.7(kJ/mol), respectively.

700T BEBE tandem mass spectrometer was high performance, especially ion intensity relatively strong and mass resolution was also good and FAB ionization was long life time available ion continuing to decide PA. But reference samples was not easy to get reference samples in case of alkali metal ions. Furthermore, the calculated affinity valued (Molecular orbital theory) are sometimes used to decide experimental affinity.

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Plenary Lecture

Tuesday, 18th September
08:00 – 08:45
Main Hall

Plenary Lecture 3: Albert J R Heck

Chair: Alma L Burlingame (UCSF, USA)

PL3-0800
08:00 – 08:45

A personal perspective on the role of mass spectrometry for protein analysis in proteomics and structural biology

Albert J R Heck[1,2]
[1]Utrecht University, Utrecht, The Netherlands, [2]Netherlands Proteomics Centre

Keywords:
mass spectrometry, stem cells, viruses

Novel aspects:
novel mass analyzers, novel quantification methods

Abstract:
Mass spectrometry is nowadays everywhere! In the life sciences mass spectrometry is heavily used especially to analyze proteins, with the aim to identify them, count their numbers present in a given living system and to characterize them in detail, which includes the analysis of protein modifications and interaction partners.
In our laboratory we focus on developing novel approaches to further analyze and characterize proteins using mass spectrometry as the primary tool. Through recent examples I will highlight how such new tools assist in the better understanding of living biological systems.
In more detail, I will describe the use of different quantification methods in MS-based proteomics to profile in-depth difference in protein expression in between embryonic and induced pluripotent stem cells and in following cellular reprogramming of fibroblasts into induced pluripotent stem cells, and how we can also use such sensitive methods to define the stemness of from murine intestine isolated low amounts of adult stem cells.
Switching gears, I will also present new data on using native mass spectrometry to structurally characterize protein assemblies, including viruses and bacterial clock systems.

Oral Session

Tuesday, 18th September

Main Hall

09:00 – 11:00

Session 11: Glycomics: From Disease Markers to Therapeutic Antibody Products

Chair: Hyun joo An (ChungNam National University, Korea)

S11-0900 [Keynote Lecture] Is high throughput glycomics possible?

09:00 – 09:40

Carlito B Lebrilla
University of California, Davis

Keywords:
Glycans, glycomics, oligosaccahrides, nanoLC

Novel aspects:
High throughput glycan analysis

Abstract:
The role of glycosylation and their biological functions whether as free oligosaccharides or glycoconjugates has been made possible by the recent advancements in the analyses of these compounds. The heterogeneity and the large structural diversity have made oligosaccharide analysis significantly more difficult than other biopolymers. The next stage of development is to achieve high throughput analysis. However, the structural elucidation of oligosaccharides remains an extremely difficult task. Recent reports reveal that the diversity of structures in a given biological system is finite and may not be large. It may be possible to create a database of structures that can be used to determine the identity of known compounds. This capability would therefore make high throughput glycomics possible. Achieving this task depends on the proper selection of chemical characteristics to identify the compound. In this presentation, nanoflow liquid chromatography retention times, accurate mass, and tandem MS is used to determine structure with a high degree of certainty. The method is used to determine the biological function of milk oligosaccharides as well as to discover glycan-based biomarkers for diseases.

Oral Session

Tuesday, 18th September

Main Hall

09:00 – 11:00

Session 11: Glycomics: From Disease Markers to Therapeutic Antibody Products

Chair: Hyun joo An (ChungNam National University, Korea)

S11-0940

09:40 – 10:00

Site specific characterization of O-GlcNAcylation on cytosolic and nuclear proteins using electron transfer dissociation mass spectrometry.

Alma L Burlingame[1], Samuel Myers[1], Jonathan C Trinidad[1], Giselle Knudsen[1], Barbara Panning[1], Agnes Thalhammer[2], Ralf Schoepfer[2]

[1]UCSF, [2]University College, London

Keywords:
O-GlcNAcylation, electron transfer dissociation

Novel aspects:
Developed a platform for separation of both O-GlcNAc and phosphopeptides from the SAME samples. Have investigated regulation of pluripotency in stem cells and signal transduction system at synapse by O-GlcNAcylation.

Abstract:
O-GlcNAcylation is the reversible, posttranslational modification of serine and threonine residues on cytosolic and nuclear proteins by β-N-acetylglucosamine. O-GlcNAc transferase and OGlcNAc-ase are the enzymes responsible for this carrying out this process, respectively. Alterations in the O-GlcNAcylation sites and stoichiometries are modulated by many stimuli, including UDP-GlcNAc levels and stress. The transferase is associated with a variety of adapter proteins, chromatin re-modeling complexes and is highly expressed in the brain. O-GlcNAcylation has been implicated in many important physiological processes including the diseases diabetes, Alzheimer and cancer. It may be particularly important in the central nervous system. OGT is present in dendrites and axon terminals ; it is also associated with microtubules. Neuron-specific deletion of OGT results in neonatal lethality due in part to abnormal neuronal development and motor deficits. Since the transferase targets protein serine and threonine residues, cross-talk with protein phosphorylation has been postulated.
We have developed a lectin based strategy to enrich native O-GlcNAcylated and multiply modified peptides from proteolytic digests of mixtures of proteins and have employed electron transfer dissociation (ETD) mass spectrometry to assign sites of modification. We have also developed a platform for separation of both O-GlcNAc and phosphopeptides from the SAME biological samples.
We have employed these methods to investigate regulation of pluripotency in mouse embryonic stem cells and the signal transduction system at the synapse. This presentation will focus on our most recent results from these investigations.
Financial support was provided by NIH NIGMS Grants P41RR001614/ P41GM103481 and P50 GM081879-02 (to ALB) .

Oral Session

Tuesday, 18th September

Main Hall

09:00 – 11:00

Session 11: Glycomics: From Disease Markers to Therapeutic Antibody Products

Chair: Hyun joo An (ChungNam National University, Korea)

S11-1000 Carbonyl trapping activity of pyridoxal 5'-phosphate in vitro and in vivo

10:00 – 10:20

Roumyana Mironova[1], Ivaylo Ivanov[2], Valentin Lozanov[3], Silvia Russeva[3], Svetlana Simova[4], Ivan Ivanov[1], Toshimitsu Niwa[5]

[1]Institute of Molecular Biology Roumen Tsanev, Sofia, Bulgaria, [2]University of Sofia St. Kliment Ohridski, Sofia, Bulgaria, [3]Medical University, Sofia, Bulgaria, [4]Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria, [5]Nagoya University Graduate School of Medicine, Nagoya, Japan

Keywords:
glycation, diabetes, carbonyl stress, vitamin-B 6

Novel aspects:
A novel physiological role for vitamin B 6 is postulated : The free, protein unbound form of pyridoxal 5 '-phosphate traps highly toxic carbonyl compounds and thus alleviates carbonyl stress.

Abstract:
Glycation is a spontaneous chemical reaction, first discovered a century ago by the French chemist Louis Camille Maillard. After him the reaction was called the Maillard reaction. In this reaction carbonyl compounds such as reducing sugars react non-enzymatically with primary amines including proteins, DNA and amino lipids to compromise their physiological functions. In the last decades it has been recognized that the Maillard reaction is implicated in pathological conditions, such as diabetic complications, renal failure and Alzheimer's disease. The deleterious consequences of the Maillard reaction have instigated the search for compounds with carbonyl trapping activity that can protect tissues from carbonyl stress and glycation damage. In previous studies we have shown that the physiologically active form of vitamin B_6, pyridoxal 5 '-phosphate (PLP) , prevents the progression of diabetic nephropathy and dialysate-induced peritoneal damage. We have also demonstrated by gas chromatography mass spectrometry that PLP scavenges the highly toxic dicarbonyl compound 3 -deoxyglucosone (3 DG) under physiological conditions *in vitro*. The aim of the present study was to solve the structure of the PLP- 3 DG reaction product and to look for its potential formation under physiological conditions *in vivo*.

In order to provide insight into the mechanism of PLP- 3 DG interaction, we have studied the reaction of PLP with three structural analogues of 3 DG. These were glycerol (propane-1,2,3,-triol (PTO)) , methylglyoxal (MGO) and D-glucose. Reactions between PLP and the model compounds including 3 DG were carried out under physiological conditions *in vitro* (37°C, pH 7.4) and the products were analyzed by high performance liquid chromatography, tandem electrospray ionization mass spectrometry coupled to liquid chromatography (LC/ESI-MS/MS) and nuclear magnetic resonance (NMR) including HRMS, ^{1}H-NMR, ^{13}C-NMR and 2 D-NMR techniques. Under these conditions PLP reacted poorly with PTO and D-glucose. In the case of PTO, glycerol-1,3-acetal ((5 -hydroxy- 4 - (5 -hydroxy-1,3-dioxan- 2 -yl) - 6 -methylpyridin- 3 -yl) methyl dihydrogen phosphate) and glycerol-1,2-acetals (*cis*- and *trans*- (5 -hydroxy- 4 - (4 - (hydroxymethyl) -1,3-dioxolan- 2 -yl) - 6 -methylpyridin- 3 -yl) methyl dihydrogen phosphate) of PLP were formed. PLP reacted slowly with D-glucose to form many isomers of aldol addition (m/z 428) and aldol condensation (m/z 410) products, the reaction involving the enediol form of D-glucose as a nucleophile and the PLP aldehyde group as an electrophile. The alpha-oxoaldehydes MGO and especially 3 DG demonstrated strong reactivity towards PLP with a yield of nearly 50% in the case of 3 DG. The reaction between PLP and MGO produced the hemiacetal 4 *H*- [1,3] -dioxino [4,5-*c*] pyridine- 4 -ol as well as PLP-MGO aldol addition product ((2 -formyl-2,4-dihydroxy- 8 -methyl-3,4-dihydro- 2 *H*-pyrano [2,3-*c*] pyridin- 5 -yl) methyl dihydrogen phosphate) and its hydrate form. In the reaction mixture of PLP with MGO we detected also an hemiacetal formed between PLP and formaldehyde, the latter resulting most probably from partial decomposition of MGO. The reaction of PLP with 3 DG resembled that of PLP with D-glucose in that both aldol addition (m/z 410) (two isomers of [(3 *S*,4*R*) -1,4,5,10a-tetrahydroxy- 3 - (hydroxymethyl) - 9 -methyl-1,3,4,4a,5,10a-hexahydropyrano [4 ',3' : 5,6] pyrano [2,3-*c*] pyridin- 6 -yl] methyl dihydrogen phosphate - major products and two isomers of { (1 *S*) - 1 - [(1 *R*) -1,2-dihydroxyethyl] -3,3a,9-trihydroxy- 5 -methyl-3,3a,9,9a-tetrahydro- 1 *H*-furo [3 ',4' : 5,6] pyrano [2,3-*c*] pyridin- 8 -yl}methyl dihydrogen phosphate - minor products) and aldol condensation (m/z 392) products were formed.

In order to evaluate the physiological significance of the PLP- 3 DG interaction, we have studied rats with streptozotocin-induced diabetes mellitus, which were treated with oral administration of PLP (600 mg/kg/day) for 16 weeks. By using LC/ESI-MS/MS we have detected PLP- 3 DG reaction products in sera and urine from the experimental animals with the aldol condensation product (m/z 392) prevailing over that of the aldol addition reaction (m/z 410) . It is noteworthy that these products were detected not only in body fluids of the PLP-treated diabetic rats but also in the control group of healthy non-PLP treated animals. Based on these experimental data we are tempted to postulate a novel physiological function for PLP : Besides its coenzyme activity, the free, protein unbound form of PLP seems to play *in vivo* a role in detoxification of the toxic alpha-oxoaldehyde 3 -deoxyglucosone and perhaps of other carbonyl compounds.

Oral Session

Tuesday, 18th September

Main Hall

09:00 – 11:00

Session 11: Glycomics: From Disease Markers to Therapeutic Antibody Products

Chair: Hyun joo An (ChungNam National University, Korea)

S11-1020
10:20 – 10:40

Bioinformatics Platform for the Glycan Marker Discovery by Mass Spectrometry

Jaehan Kim[1], Hyunjoo An[1], Carlito Lebrilla[2]

[1]Chungnam National University, Daejeon, Korea, [2]University of California, Davis, USA

Keywords:
Glycomics, Biomarker, Two-dimensional data analysis, Glycan correlation

Novel aspects:
We sugget the bioinformatics platform for the high throughput analysis of MS dataset for biomarker diacovery. In addiion, the glycan correlation was emplyed to validate the glycan marker

Abstract:
Despite the fast growing interest, mass spectrometry is still not well accepted as a primary discovery tool, particularly in glycobiology. Recent advancement of mass spectrometric instrument enable to provide high accuracy of molecular mass of glycans, however, the wide range of their stereochemical and structural diversities hampers to conclude the glycan information that observed from unknown samples. Despite the rapid advancement, the elucidation of the structural (or compositional) information of glycans by MS/MS tandem mass still necessitate the labor intense work and exhibit various limitations. Lack of bioinformatical platform for the analysis of glycan causes the uncertainty in the data interpretation obtained from the mass spectrometry and makes the conclusive judgment of experimental observation difficult. Particularly, the statistical tools used for proteomics such as PCA, ANOVA, or hierarchical cluster analysis are easily employed without the consideration for the unique biological properties of glycans and the statistical characteristics of glycan profiling by MS. The lack of an appropriately customized bioinformatics platform for the analysis of MS data hampers the progress in glycan marker discovery. To overcome the current limitations, we employed two-dimensional analysis using frequency and intensity domain after a series of data filtration. Glycan correlation was applied to evaluate the biological relevance as a glycan as well as enabling a comprehensive glycomics that can examine the glycans and modified glycans. With this approach we have found a set of potential glycan markers with the high sensitivity and high specificity in patient sera with ovarian cancer.

Oral Session

Tuesday, 18th September

Main Hall

09:00 – 11:00

Session 11: Glycomics: From Disease Markers to Therapeutic Antibody Products

Chair: Hyun joo An (ChungNam National University, Korea)

S11-1040
10:40 – 11:00

Characterization of Elastin Degraded Peptides in Chronic Obstructive Pulmonary Disease (COPD)

Yong Y Lin[1], Toyonobu Usuki[2]

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Keywords:
Elastin degraded peptides, COPD, Biomarkers, LC-ESI/MSMS, Peptidpmic

Novel aspects:
Elastn degradation which occurs in prevalent diseases have been studied. LC-MS/MS characterization of elastin degraded peptides was performed to study disease development and search for potential biomarkers.

Abstract:
Characterization of Elastin Degraded Peptides in Chronic Obstructive Pulmonary Disease (COPD)

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Elastin is a highly crosslinked insoluble protein responsible for elasticity and resilience in various tissues including arterial blood vessels, lung, and the skin. The degradation of elastin-containing tissues occurs in several widely prevalent diseases, such as pulmonary emphysema, chronic obstructive pulmonary disease, cystic fibrosis, atherosclerosis, aortic aneurysm, etc. By utilizing mass spectrometric characterization we have been investigating the degradation of lung elastic tissues involved in chronic obstructive lung disease (COPD). COPD is well recognized as one of the major health problem in the world. In the US it has moved to the third leading cause of death.

An early insight into the mechanisms leading to lung alveolar destruction in patients with COPD is that lung elastin is a target for protease degradation by cellular elastases. Elastin is a highly crosslinked insoluble protein formed by condensation of lysyl residues in the soluble precursor, tropoelastin (786 amino acids), which can be degraded into soluble peptide fragments by the elastolytic enzymes produced by neutrophils and macrophages. Two pyridinium compounds, desmosine (D) and isodesmosine (I) were known as the major crosslinking molecules of insoluble elastin. We have developed a specific and sensitive LC-MS/MS using a novel d_4-desmosine as the internal standard, which can measure D and I in biofluids and has shown to be an effective biomarker in COPD.

We have also studied degradations of lung elastin into peptide fragments by the specific proteases neutrophil elastase and macrophage elastase, two major elastases involved in lung elastin degradation in COPD. Utilizing peptidomic LC-MS/MS we have characterized over 50 of elastin degraded peptides (EDPs), some of them have been identified in plasma of COPD patients. Inflammation mediated by T cells in the lung has been considered a possible perpetuating disease mechanism in COPD. These EDPs generated during lung injuries may modulate inflammation and lead to the progression of COPD. The detection and characterization of such peptides will allow us to investigate further enzymatic degradation process involved in pathogenesis in COPD and search for effective peptide biomarkers and target for therapy.

In addition, we have synthesized and studied LC-MS/MS fragmentography of several desmosine peptides as models for the crosslink structure of elastin.

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desmosine that crosslinks elastin. *Chem Commun* 2012；48：3233-3235.

Oral Session

Tuesday, 18th September — Room A

09:00 – 11:00

Session 12: On-site Mass Spectrometry -Miniaturized Instruments and Allied Technologies-

Chair: Shuichi Shimma (National Cancer Center Research Institute, Japan)

S12-0900
09:00 – 09:40

[Keynote Lecture] In-vivo, Real-time Identification of Tissues in Human Surgical Environment by Rapid Evaporative Ionization Mass Spectrometry

Zoltan Takats[1], Julia Balog[2], James Kinross[1], Christian Schafer[1], Laura Muirhead[1], Ottmar Golf[4], Laszlo Sasi-Szabo[3], Balazs Dezso[3], Kirill Veselkov[1]

[1]Imperial College, London, UK, [2]Medimass Ltd., Budapest, Hungary, [3]University of Debrecen, Debrecen, Hungary, [4]Justus Liebig University, Giessen, Germany

Keywords:
ambient ionization, ion transfer, lipidomics

Novel aspects:
Presentation of a mass spectrometric method for the in-vivo identification of tissues during surgical interventions, together with the results of first clinical study involvig 250 subjects.

Abstract:
Rapid identification of biological tissues is a long-standing problem on various fields of interventional medicine, with special regard to cancer diagnostics and cancer surgery. While healthy tissue types (including benign tumors) show complete anatomical separation, malignant proliferations infiltrate surrounding healthy tissue parts, making visual localization extremely hard, or even impossible. While histological techniques provide the ultimate solution for the cellular-level identification of cancer cells, the approach is extremely complex and time consuming. Nevertheless, accelerated version of histopathology (so-called 'frozen section' method) is widely used for the intraoperative characterization of tissue samples removed from the surgical area. Since frozen section histology is less reliable than the traditional approaches, and the accelerated procedure still takes approx. 30 minutes for a single sample, there has been ongoing research for the development of more accurate and faster methods.
Molecular spectroscopy techniques including IR, Raman, solid state NMR and mass spectrometry have been used for the characterization of intact biological tissues and showed enormous potential for the differentiation of tissues with various histologies, including multiple different types of cancer. While all of these techniques are able to provide rapid information, the underlying experimental setups have not allowed the utilization of these techniques in surgical or diagnostic environments (e.g. in case of mass spectrometric imaging of tissue sections) .
Rapid Evaporative Ionization Mass Spectrometry is based on the observation that electrosurgical dissection of vital tissues involves the ionization of various tissue constituents, with special emphasis on membrane lipids. Electrosurgical methods employ electric current for the rapid heating and evaporation of tissue material and they are widely used both for dissection and coagulation on practically all fields of surgery. Hence, the direct combination of electrosurgery with mass spectrometry provides a tissue identification methodology, where the tissue manipulation part is already widely used by surgeons and fully approved from regulatory point of view. Electrosurgical handpieces have been coupled to mass spectrometers using flexible polymer tubing and Venturi air-jet pump. The experimental setup allows the transfer of ionic species from surgical site to mass spectrometer installed in the operating theatre. Mass spectrometers were equipped with special, noise insulated airtight chassis for surgical applications.
REIMS data of human tissues were found to show high histological specificity and excellent patient-to-patient reproducibility. While the detected complex lipid-type species generally occur in most tissue types, their distribution pattern is unique for the given histological types. Spectral data was analyzed using multivariate statistical approaches including principal component analysis and linear discriminant analysis. Based on these approaches, a rapid tissue identification system was implemented, which is capable of the identification of unknown tissues within 1 sec, during surgical interventions. The technique has been tested in case of gastrointestinal adenocarcinoma and its liver metastases, involving 250patients. Tumor tissue has been identified successfully in all cases either in-vivo or using fresh ex-vivo tissues. Keeping false negative rates at 0 %, false positive rate < 0.5 % has been achieved.
While the tissue identification process requires large number of histologically assigned authentic spectra, collection of database entries in a parallel fashion with histopathological examination is time and sample consuming process. Nevertheless, more than 40,000 individual database entries were collected and stored in the REIMS database. Since imaging mass spectrometric methods are able to provide histologically resolved data on tissue lipidome, we have developed a spectral pre-processin galgorithm to bring DESI/MALDI imaging and REIMS data to a statistically comparable level. The combination of different mass spectrometric analysis modalities results in 3 orders of magnitude faster data collection rate and significantly improved histological accuracy.

Oral Session

Tuesday, 18th September

Room A

09:00 – 11:00

Session 12: On-site Mass Spectrometry -Miniaturized Instruments and Allied Technologies-

Chair: Shuichi Shimma (National Cancer Center Research Institute, Japan)

S12-0940
09:40 – 10:00

Miniaturization of a Toroidal Ion Trap Mass Analyzer Composed of Cylindrical Electrodes

Daniel E Austin, Nicholas Taylor, Jessica Higgs
Brigham Young University, Provo, Utah, USA

Keywords:
toroidal, ion trap, quadrupole

Novel aspects:
first demonstration of toroidal ion trap using cylindrical electrodes

Abstract:
Simplification of electrode geometry allows ion traps to be miniaturized without tolerance complications associated with machining hyperbolic surfaces. Cylindrical and rectilinear ion traps exemplify this process : each uses electrodes that are geometrically simpler than those used in the quadrupole and linear ion traps, respectively. A challenge of accomplishing this process with toroidal ion traps is the asymmetry that is needed to counteract the curvature of the trapping region. We present a novel toroidal ion trap design employing cylindrical electrodes. Asymmetric overlap of the electrodes compensates for the curvature of the toroidal trapping region. Mass spectra of several organics demonstrate instrument performance, including mass resolution better than that reported in other toroidal devices.

A full-size (r_0 = 6 mm) prototype device was constructed, consisting of four electrodes : one inner cylinder (ground) , one outer cylinder (AC) , and two end-cap plates (RF) . Mass analysis is accomplished using resonance ejection and a ramp of the applied RF amplitude. Mass-analyzed ions are radially ejected to a single point at the center of the device by an ac signal applied to the outer cylinder electrode. Headspace vapor samples were ionized in situ using an electron gun aimed at a small slit in the outer cylinder electrode. Higher-order components of the electric field were optimized using SIMION. Several different values of higher-order field components were studied experimentally by varying the spacing between the two end-cap plates (RF) . Both forward and reverse scans were explored.

For toluene samples, the 91-92 peaks are baseline resolved, with mass resolution of 0.32. Mass resolution as a function of the RF end-cap spacing is best when the octopole-component of the trapping field is slightly positive, +0.4 % , and gets progressively worse above and below this value. Evaluation of both forward (mass selective instability scan) and reverse (resonance ejection scan) scans give fairly similar resolution for a given octopole value with the optimum being at +0.4 % for the reverse scan with resonance ejection. Simulations indicate that ejection efficiency is close to unity : all ions are ejected radially toward the center, and none are lost in the opposite direction. Tandem mass analysis (MS^2) was also demonstrated using this device.

Under current development are two miniaturized versions of this device (r 0 = 1 and 2 mm) , in which the RF and AC electrodes are made using sheets of stainless steel cut using wire EDM. This approach greatly reduces machining costs, and also allows uniformity of trapping dimensions along the toroidal trapping region. Because the major toroidal radius is unchanged from the full-size version, the trapping capacity is not significantly reduced in these miniaturized versions. In addition, an ion guide is integrated on the trap, forming a “p “ shape, to facilitate introduction of ions into the trap. Simulations show that this eliminates the RF barrier for ion introduction.

Oral Session

Tuesday, 18th September

Room A

09:00 – 11:00

Session 12: On-site Mass Spectrometry -Miniaturized Instruments and Allied Technologies-

Chair: Shuichi Shimma (National Cancer Center Research Institute, Japan)

S12-1000
10:00 – 10:20

Auto-sampling Explosives Trace Detection System using Mass Spectrometry

Yuichiro Hashimoto, Hisashi Nagano, Yasuaki Takada, Yasutaka Suzuki, Hideo Kashima, Masakazu Sugaya, Yasunori Doi, Koichi Terada, Minoru Sakairi

Hitachi, Ltd. Central Research Laboratory

Keywords:
Explosives Trace Detection, Cyclone-type Concentrator

Novel aspects:
Development of a novel auto-sampling explosives trace detection system using mass spectrometry that achieved high-throughput detection within a few seconds for various types of explosives.

Abstract:
Introduction
Due to the ease of obtaining procedures for making explosive devices via the Internet, the threat of improvised explosive devices (IEDs) has increased in many countries. Since explosives trace detector (ETD) is a powerful tool to help prevent terror attacks, many types of ETDs are being developed currently. We developed a high-throughput auto-sampling explosives trace detector (ETD) portal using mass spectrometry [1]. The ETD portal achieved high-speed detection of volatile explosives within a few seconds without human hands. We also reported a false-positive rate of less than 0.1% when the prototype detector was deployed in at ticket gates at train stations in Japan [2]. In this paper, I'll review our recent developments of a novel auto-sampling ETD system, which achieved less volatile explosives detection using a cyclone particle concentrator.

Method
To extend the analysis targets to less volatile explosives such as TNT, PETN, and RDX, particle sampling as well as vapor sampling is required. We've been developing a novel particle sampler using a cyclone-type particle concentrator that can quickly and effectively collect and concentrate explosive particles that are attached to objects. This sampler consists of detachment, evacuation, condensation, and heating components. First, explosive particles on a card are detached by air jet pulses and then collected by evacuation. Particles are collected by a cyclone particle separator, where only particles are collected near the bottom of the container. The collected particles are heated, and then the explosive vapor is introduced into a mass spectrometer.

Preliminary data
We tested a prototype of the particle sampler using NIST SRM 2905, trace particulate explosive simulants. All the signals from the ug-level explosive particles, which included a percent ng level of TNT and RDX, were detected within a few seconds after an air jet pulse was injected from a spray nozzle onto a plastic card. An appropriate evacuation flow rate is over 50 L/min because anything lower will not be sufficient to collect the detached particles in the sample collector. However, the typical flow rate into a mass analyzer is about 1 L/min. Therefore, the cyclone separator, which is positioned between the sample collector and the mass spectrometer, is used to condense the explosive vapor from the particles to a volume 50 times smaller compared to the case without it. The collection efficiency of the cyclone separator is over 50%, which means the sensitivity was enhanced by more than 25 times using the cyclone separator compared to the case without it. After a TNT signal was detected, the signal level returned to the background level in less than 20 seconds. The recovery time strongly depends on the heating temperature of the particle heater.

This work was supported by "R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society ", Strategic Funds for the Promotion of Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References
1) Y. Takada et al., *Rapid Commun. Mass Spectrom.* 2011；25 (17)：2448.
2) Y. Hashimoto et al, "Field test evaluation of a walkthrough portal detector of improvised explosive devices at a train station ", *Trace Explosives Detection Workshop*, April, 2011, Portland.

Oral Session

Tuesday, 18th September

Room A

09:00 – 11:00

Session 12: On-site Mass Spectrometry -Miniaturized Instruments and Allied Technologies-

Chair: Shuichi Shimma (National Cancer Center Research Institute, Japan)

S12-1020

10:20 – 10:40

Rapid Screening Chemicals in the Liquids and Solids via Mobile Ambient Mass Spectrometry (MAMS)

Min-Zong Huang, Chu-Nian Cheng, Hung Su, Jentaie Shiea

National Sun Yat-Sen University, Kaohsiung, Taiwan

Keywords:

Mobile Ambient Mass Spectrometry ; thermal desorption ; miniaturized mass spectrometry

Novel aspects:

Mobile ambient mass spectrometry was used to rapidly screen chemicals in the liquids and solids samples.

Abstract:

A mobile ambient mass spectrometry (MAMS) was developed and constructed for rapid characterization of chemicals in the liquids and solids. The MAMS system is comprised of a commercial mass spectrometer equipped with an ambient ionization source, gas generator, and power supply. The MAMS was set on a moving cart for expanded mobility ; shock absorbers were added to protect the system from damage when the entire apparatus was on the move. Three steps are usually involved ina typical MAMS analysis - sampling, thermal desorption, and electrospray ionization. The liquid or solid sample attached on a metal probe was inserted in a preheated oven. The analytes in the sample were subsequently desorbed and conducted to the tip of a capillary where ions and charged droplets were continuously generated by electrospraying an acidic methanol solution. The desorbed analytes reacted with the charged methanol species or fused with the charged solvent droplets to produce analyte ions. The analytes could be protonated by the ion-molecule reactions between analytes and solvent ions, or fused in the charged solvent droplets and then electrospray ionized. Since the total analytical time neededis only three seconds per sample, a high-throughput screening strategy can be made by this technique.

The MAMS system is integrated delicately on a golf cart ; therefore, its moveability is non-restricted that the analysis can be performed wherever is needed. In addition, because the conventional mass spectrometer is employed, the MAMS system has wider applicability and higher specificity than that of the portable MS. This feature has broadened its practical use in on-site analysis, and made itself as a powerful analytical means in the areas of public safety, environmental protection, and pollution monitoring.

S12-1040
10:40 – 11:00

Development of portable vacuum ultraviolet single photon ionization mass spectrometer for trace measurement of volatile organic compounds

Yukio Yamamoto[1], Hiroyuki Yamada[2], Kenichi Tonokura[1]

[1]The University of Tokyo, Chiba, Japan, [2]National Traffic Safety and Environmental Laboratory, Tokyo, Japan

Keywords:
photoionization, time-of-flight mass spectrometer, volatile organic compounds, vehicle emission

Novel aspects:
Portable VUV-SPI-TOFMS was applied to real-time measurement of VOCs in the vehicle exhaust gas.

Abstract:
Volatile organic compounds (VOCs) play an important role in the tropospheric chemistry in terms of production of ozone and the organic aerosols. It is necessary to understand the composition and concentration of numerous VOCs in the environment since the mechanisms for ozone and organic aerosols formation are different for every VOC. New techniques that can perform in-situ and real-time measurement of each VOC are needed for the identification of the sources and the distributions of VOCs. Time-of-flight mass spectrometry (TOFMS) is a suitable technique for quantitative and qualitative analysis of trace VOCs in an environment. The advantages of MS for air analysis are excellent sensitivity and real-time response. TOFMS has a merit of its compact size. Single photon ionization (SPI) using vacuum ultraviolet (VUV) light is a threshold ionization method for analytical mass spectrometry. VUV-SPI is also a soft ionization technique that can be adjusted to be nearly fragmentation free and the molecular ion peaks of most organic molecules can be observed. For real-time measurement of VOCs with high sensitivity, we have developed VUV-SPI-TOFMS[1].
The compact VUV-SPI-TOFMS that we developed is 0.5 m × 0.5 m × 0.5 m in size and about 30 kg in weight, excluding the power supply for the Nd：YAG laser. Ionization is conducted by photoionization at 10.5 eV (wavelength：118 nm) VUV light generated by the 9th harmonics of a Nd：YAG laser. Accelerated ions are introduced to field-free drift region and are reflected by reflector. The ion signals are detected by microchannel plate. The TOF mass spectra can be recorded in real time or stored on the hard disk to be analyzed by home-written LabVIEW software programs. We achieved a limit of detection of 8, 11, and 18 parts per billion by volume (ppbv) for benzene, toluene, and chlorobenzene, respectively, with an integration time of 5 s (100 measurements averaging) . A mass resolution of 800 at 112 *m/z* was obtained in a 0.35 m long reflectron TOFMS instrument[1].
The measurements of vehicle exhaust gases were demonstrated[2]. The vehicle emission is one of the major sources of VOCs in atmosphere. Because the vehicle exhaust constantly changes in synchronization with engine operation, real-time measurement is required to analyze the species in exhaust gases. The exhaust gases were sampled in an exhaust pipe of tested vehicles driven on a chassis dynamometer. The TOF mass spectra of the sampled gas were averaged and recorded every 1 second (20 single-shot spectra) . The obtained signals were converted into concentration profiles using premixed standard gas. Emission profiles of VOCs from both gasoline and diesel engine vehicles were measured. Alkylbenzens, alkenes, alkanes, and dienes were measured in addition to ppmv-level aromatics such as benzene and toluene in the vehicle exhaust gases. The changes in concentrations of VOCs in the exhaust gases were different between gasoline and diesel engine vehicles. We obtained the mass spectra of the exhaust during each driving time and the temporal profiles of every *m/z* in one measurement. Furthermore, from the measurements of hydrocarbons using the VUV-SPI-TOFMS, we obtained time profiles of the emitted weights of VOCs and their total emissions. We have shown that VUV-SPI-TOFMS is useful for real-time and simultaneous measurements of VOCs in the vehicle exhaust with high sensitivity and real-time response.

References：
[1] K. Tonokura, N. Kanno, Y. Yamamoto, and H. Yamada, *Int. J. Mass Spectrom.*, 290, 9-13 (2010) .
[2] Y. Yamamoto, Y. Kambe, H. Yamada, and K. Tonokura, *Anal. Sci.*, 28, 385-390 (2012) .

Oral Session

Tuesday, 18th September

Room B-1

09:00 – 11:00

Session 13: Accelerator Mass Spectrometry

Chair: Hiroyuki Matsuzaki (The University of Tokyo, Japan)

S13-0900

09:00 – 09:40

[Keynote Lecture] Accelerator Mass Spectrometry - analysis of the rarest atom species for earth and environmental science

Peter Steier[1]

[1]University of Vienna, Faculty of Physics

Keywords:
Accelerator mass spectrometry, Anthropogenic nuclides, Beryllium-10, Iodine-129, Uranium-236, Ocean Tracers, Fission products, Isobar separation

Novel aspects:
Many new AMS facilities emerge for the "traditional" AMS isotopes Be-10, C-14, Al-26, Cl-36, and I-129, while the development proceeds towards more efficient measurements with always smaller machines.
Additionally, in the next years, improved isobar separation will allow to extend the AMS technique to the middle mass range from 60 to 200 amu. Since many long-lived anthropogenic and natural fission products are present in the environment, many new applications will show up.

Abstract:
Accelerator Mass Spectrometry (AMS) excels over other methods of analytical isotope research by its extraordinary abundance sensitivity, i.e. the ability to separate out the rarest atom species from abundant stable isotopes. This makes the method the perfect fit for long-lived radionuclides with half-lives between 1 kyr and 100 Myr, of both natural and anthropogenic origin. During the last 30 years, AMS has made significant contributions to practically all fields of science.
Dating based on natural C-14 for archaeology and palaeontology is still one major application. With increasing precision, radiocarbon dating starts to compete with traditional historical dating, e.g. connected to the ancient Thera eruption in the Eastern Mediterranean Sea. Anthropogenic C-14, produced by the atmospheric nuclear weapons tests, allows for so-called bomb-peak dating with a precision of about 1 year. This development has fallen on fertile ground in biomedicine. Dating of human tissue and especially DNA reveals new insights into turnover rates and regeneration. Pharmaceutical research benefits also from so called "microdosing" which studies the human metabolism with C-14 labeled, but nonhazardous small amounts of new substances. New dedicated C-14 AMS systems usually have reduced size and complexity, providing competitive performance with a footprint of only a few square meters.
A complementary trend to larger size accelerators is observed for the upcoming facilities aiming at the other "traditional" AMS isotopes Be-10, Al-26, Cl-36, and I-129. Their detection in surface rocks or in natural archives like ice cores allows to investigate the processes shaping our planet, and to assess their time scales, which is the basis for the prediction of the impact of anthropogenic activities on a global scale. The new facilities are often designated as working horses for earth and environmental science, and are not necessarily affiliated at physics departments.
Recently, U-236 and the plutonium isotopes from global fallout have demonstrated their potential as environmental tracers. U-236 shows conservative behavior in the ocean. Pu, on the other hand, binds to soil particles, and can thus serves as a tracer for sediment transport. Both uranium and plutonium can be used to detect releases of nuclear fuel.
The main technical challenge for AMS is imposed by stable isobars from other elements, which are always present at the ppm level even in purified samples. While the higher energy of AMS allows their suppression via the different energy loss in matter for the lighter masses, even the largest machines cannot generally separate isobars above Fe-60. Many isotopes in the middle mass range with suitable half-life are present in the environment as natural and anthropogenic fission products. New methods based on chemical reaction cells or laser detachment are developed to remove the interfering isobars. Once successful, a whole new spectrum of applications will be opened.

Oral Session

Tuesday, 18th September

Room B-1

09:00 – 11:00

Session 13: Accelerator Mass Spectrometry

Chair: Hiroyuki Matsuzaki (The University of Tokyo, Japan)

S13-0940

09:40 – 10:00

Small scale radiocarbon dating and its applications to understand Antarctic ice sheet

Yusuke Yokoyama[1], Yosuke Miyairi[1], Suga Hisami[2], Matsuzaki Hiroyuki[3], Ohkouchi Naohiko[2]

[1]University of Tokyo, Chiba, Japan, [2]JAMSTEC, Yokosuka, Japan, [3]University of Tokyo, Tokyo, Japan

Keywords:
AMS, Radiocarbon, Small Scale, Antarctica

Novel aspects:
Small scale radiocarbon dating on compound specific measuremens conducted by AMS allows us to understand Earth's surface dynamics. Antarctic ice sheet melting history is clearly depicted.

Abstract:
Recent advancement of accelerator mass spectrometry (AMS) enables us to conduct small scale radiocarbon dating. This allows us to measure compound specific measurements for understanding dynamics of Earth surface systems in detail. We have developed AMS measurement techniques for small- scale samples ranging from 0.01 to 0.10 mg C (Yokoyama et al., 2010) with a new type of MC- SNICS ion source system (Southon and Santos, 2007) . We can generate 4 times higher ion beam current intensity for ultra-small samples by optimization of graphite position in the target holder with the new ionizer geometry. CO 2 gas graphitized in the newly developed vacuum line is pressed to a depth of 1.5 mm from the front of the target holder. This is much deeper than the previous position at 0.35 mm depth. We measured 12C 4 + beam currents generated by small standards and ion beam currents (15-30 μA) from the targets in optimized position, lasting 20 min for 0.01 mg C and 65 min for 0.10 mg C. We observed that the measured 14C/12C ratios are unaffected by the difference of ion beam currents ranging from 5 to 30 μA, enabling measurement of small size samples with high precision. Examination of the background samples revealed 1.1 μg of modern and 1 μg of dead carbon contaminations during target graphite preparation. We make corrections for the contamination from both the modern and background components. Reduction of the contamination is necessary for conducting more accurate measurement.
In this presentation, we will introduce our system at our lab and recent developments regarding further efforts of reducing background. Then present some geological applications using sediment samples obtained around Antarctica coupling also with 10Be measurements.

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Oral Session

Tuesday, 18th September

Room B-1

09:00 – 11:00

Session 13: Accelerator Mass Spectrometry

Chair: Hiroyuki Matsuzaki (The University of Tokyo, Japan)

S13-1000

10:00 – 10:20

Beryllium 10 analysis for the Dome Fuji ice cores and cosmic-ray stratigraphy

Kazuho Horiuchi[1], Shota Suguchi[1], Kensuke Suda[1], Tomoko Uchida[2], Takahiro Aze[3], Yusuke Yokoyama[4], Yasuyuki Muramatsu[5], Hiroyuki Matsuzaki[4], Hideaki Motoyama[6]

[1]Hirosaki University, Hirosaki, Japan, [2]IAA, Shirakawa, Japan, [3]Tokyo Institute of Technology, Tokyo, Japan, [4]The University of Tokyo, Tokyo, Japan, [5]Gakushuin University, Tokyo, Japan, [6]National Institute of Polar Research, Tokyo, Japan

Keywords:
Beryllium 10, Cosmic ray, Paleointensity, Stratigraphy

Novel aspects:
A novel long-term record of cosmogenic beryllium 10 from Antarctica that serves as a proxy of the paleo-cosmic-ray flux and as a standard curve for cosmic-ray stratigraphy.

Abstract:
Long-term records of cosmogenic radionuclides (such as ^{10}Be, ^{14}C, and ^{36}Cl) provide a clue to understand the history of cosmic ray variations and climate-driven changes in the fallout/depositional systems in the atmosphere, land and ocean. They can be also utilized for radiometric and/or stratigraphic dating back to the past ten million years.

We have been analyzing the cosmogenic radionuclide ^{10}Be in polar ice cores by employing one of the state-of-the-art systems of Accelerator Mass Spectrometry (AMS) in Japan. As a good example of the results of our project, we show here a 300-kyr record of ^{10}Be in the Dome Fuji ice cores with a time resolution of 1 kyr. The Dome Fuji ice cores were drilled at the Dome Fuji station (77° 19'S, 39° 42'E), inland East Antarctica. The concentration of ^{10}Be in the ice samples was determined by using an AMS system at The University of Tokyo (MALT-AMS system). The measured concentration was converted to the flux by multiplying the snow accumulation rate estimated by Parrenin et al. (2007). The chronology of the cores is based on a direct and precise orbital tuning with the variations in the ratios of O_2 and N_2 in air trapped in the ice cores (Kawamura et al., 2007).

The ^{10}Be flux over the past 300 kyr recorded in the Dome Fuji ice cores showed distinct increasing peaks at around 41, 114, and 189 ka, which are correlative to the well-known geomagnetic excursions Laschamp, Blake, and Iceland Basin, respectively. Empirical and semi-quantitative estimation of geomagnetic paleointensity from the ^{10}Be flux indicates that most of the variations in ^{10}Be were attributable to the variations in cosmic-ray intensity modulated by geomagnetic effects for incoming cosmic-ray particles. This fact strongly suggests that our ^{10}Be record properly serves as a proxy of the paleo-cosmic-ray flux and as a standard curve for the stratigraphic correlation based on the cosmic-ray variations (i.e. as a standard curve for cosmic-ray stratigraphy). In this presentation, we will also explore the possibility of the cosmic-ray stratigraphy using our 300-kyr record of the ^{10}Be flux.

References :
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Oral Session

Tuesday, 18th September

Room B-1

09:00 – 11:00

Session 13: Accelerator Mass Spectrometry

Chair: Hiroyuki Matsuzaki (The University of Tokyo, Japan)

S13-1020

10:20 – 10:40

Quantifying soil formation and sediment yield in mountains watersheds using terrestrial cosmogenic nuclides

Yuki Matsushi[1], Hiroyuki Matsuzaki[2]

[1]Kyoto University, Uji, Japan, [2]The University of Tokyo, Tokyo, Japan

Keywords:
terrestrial cosmogenic nuclides, sediment yield, soil production, denudation rate, geomorphology

Novel aspects:
Advances in sensitivity and accuracy in measurements, and evolution in methodology in applying isotopes in mountain geomorphology.

Abstract:
This report overviews principle and potential for use of terrestrial cosmogenic nuclides in mountain geomorphology, especially for quantification of earth surface processes. Technological advance in accelerator mass spectrometry enables us to measure cosmogenic nuclides in rock minerals such as ^{10}Be and ^{26}Al in quartz, and ^{36}Cl in calcite, which provide exposure ages or denudation rates of land surfaces. In recent decades, the nuclide analysis offers not only the minimum exposure age or maximum denudation rate of a single rock surface, but spatially-averaged denudation rate of a watershed, soil production rates in hill slopes, and also information for reconstructing basin development in mountainous regions under humid temperate climates. We present example applications in Japan, which are determination of sediment yield from watersheds by analyzing fluvial sand, and soil formation and transport by analyzing saprolite (weathered bedrock beneath soil layer) in mountain catchments underlain by granitic rocks. These applications offer potential usage of terrestrial cosmogenic nuclides for quantitative assessment of mountain hazards with comprehensive understanding of long-term sediment dynamics in mountainous terrains.

S13-1040

10:40 – 11:00

Iodine isotope system in environment: Natural system, anthropogenic system and influence from NPP accident

Hiroyuki Matsuzaki[1], Maki Honda[2], Yasuto Miyake[1], Takeyasu Yamagata[2], Hironori Tokuyama[1], Yasuyuki Muramatsu[3]

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Keywords:
Iodine-129, Iodine, Accelerator Mass Spectrometry, Cosmogenic radio nuclide, Fukushima Dai-ichi

Novel aspects:
Natural equilibrium iodine isotopic ratio in marine environment was found to be 2 x10-13, which is lower by one order previously proposed.

Abstract:
Iodine isotope system, concerning with wide ranges of isotopic ratio ($10^{-14}$to 10^{-6} as $^{129}I/^{127}I$) has great potential to investigate various aspects of earth environmental systems. Before 1950's there had exist only "natural iodine isotope system " where ^{129}I had been provided by two sources, cosmogenic and fissiogenic. The half life of the ^{129}I is 1.57 $x10^{7}$ yr which is, though negligibly short compared to the earth history, much longer than ordinary material circulation in the earth system such as marine circulation. The iodine isotopic ratio in a certain compartment is determined by the balance between the turnover time and the production rate. Generally this balance is different with site to site. But if the circulation is well quick between different sites, the isotopic ratio is identical everywhere. If this hypothesis is true, or within the region this is true, the dating of the closed system should be possible from the isotopic ratio. The earth surface environment, especially marine, seems to be the case. The iodine isotopic ratio of $^{129}I/^{127}I$ = 1.5 x 10^{-12} had been proposed and used as the initial value for dating (J. Moran, 1998) . It was shown that, using this initial ratio, the age of the methane hydrate could be determined (U.Fehn, 2000) . Afterwards the ages of many geologic samples especially iodine containing fluids and sedimentary rocks have been determined. However some samples showed discrepancy between the iodine isotopic age and geological determined age.
To examine whether the natural iodine is fully mixed in the ocean and whether the initial value previously proposed ($^{129}I/^{127}I$ = 1.5 x 10^{-12}) is valid enough, we measured depth profiles from surface to bottom of $^{129}I/^{127}I$ratio for seawater samples collected from several points in the Indian ocean. The results showed significantly low $^{129}I/^{127}I$ ratio (around 2 $x10^{-13}$) in the middle to deep layer. According to our results, the initial value should be one order lower than that of previously proposed. Serious difficulty, then, arises for the interpretation of samples having isotopic ratio higher than 2 $x10^{-13}$ but lower than 1.5 x 10^{-12}. It should be interpreted as the anthropogenic contamination.
The other possibility is that the iodine is not mixed enough between different sites. To examine this issue, the elemental iodine dynamics (circulation path, rate, turnover time) should be examined for several compartments in the earth surface environment including atmosphere, soil, fluvial system, underground water, and marine environments. In this context, anthropogenic ^{129}I can be used as the tracer of the iodine dynamics. After human began to utilize the nuclear power, ^{129}I as a byproduct of the fission nuclear reaction entered to the earth surface environment overwhelming the naturally produce ^{129}I. As a result, the isotopic ratio of the surface environment increased by several orders. The spatial distribution of $^{129}I/^{127}I$ tells us the iodine dynamics in the environment. The accident at Fukushima Dai-ichi nuclear power plant also made a spike of ^{129}I onto the land surface, which provides an useful tracer for observation of the iodine diffusion process. As one of important sites for iodine reservoirs we have been focusing on the soil. The depth profile of $^{129}I/^{127}I$ ratio in the soil indicates that there are at least two different modes of moving way, quick diffusion and slow movement.

Oral Session

Tuesday, 18th September

Room D

09:00 – 11:00

Session 14: Ion-surface Collisions: Collision-induced Dissociation and Soft Landing

Chair: Jean H Futrell (Pacific Northwest National Laboratory, USA)

S14-0900

09:00 – 09:40

[Keynote Lecture] Ion-Surface Collisions in Mass Spectrometry: Activation, Dissociation and Soft-Landing

Julia Laskin

Pacific Northwest National Laboratory, Richland, WA, USA

Keywords:
surface-induced dissociation, soft-landing, reactive landing, kinetics, self-assembled monolayer surfaces

Novel aspects:
Overview of the field

Abstract:
Collisions of hyperthermal (1-200 eV) complex ions with surfaces are utilized in mass spectrometry studies for structural characterization of large molecules and for highly selective modification of surfaces. Two major processes are dominant in this range of collision energies : reactive and non-reactive scattering of ions and ion loss on the surface as a result of neutralization or soft-landing of projectile ions. Ion activation by collisions with a surface followed by surface-induced dissociation (SID) of vibrationally excited ions offers unique advantages both for analytical applications and for fundamental studies of gas-phase fragmentation of complex ions. Measuring the time dependence of surface induced dissociation (SID) of complex ions with specially prepared surfaces as a function of ion kinetic energy uniquely defines the energetics, dynamics and mechanisms of fragmentation of complex molecules in the absence of solvent. This approach provides important information on the strength of covalent bonds in complex molecules and has been recently extended to understanding factors that determine dissociation of non-covalent complexes. Ion capture on surfaces is the principal competing reaction channel to scattering and SID. Depending on the experimental conditions, ions are captured as intact charged species, exchange charges with the substrate, evaporate or react with functional groups on the surface. Many studies demonstrated that preparatory mass spectrometry is a unique tool for highly selective preparation of novel materials and for obtaining molecular level understanding of the effect of the surface on the structure, charge state, and conformations of trapped ions. Several examples of controlled deposition of complex ions on self-assembled monolayer (SAM) surfaces demonstrating the utility of preparatory mass spectrometry for highly-selective preparation of biological and catalytic surfaces will be presented and factors affecting the competition between physical and chemical processes accompanying ion deposition will be discussed.

Oral Session

Tuesday, 18th September

Room D

09:00 – 11:00

Session 14: Ion-surface Collisions: Collision-induced Dissociation and Soft Landing

Chair: Jean H Futrell (Pacific Northwest National Laboratory, USA)

S14-0940 Surface-induced Dissociation of Non-covalent Macromolecular Complexes

09:40 – 10:00

Vicki H Wysocki[1,2], Mowei Zhou[1,2], Anne Blackwell[2], Royston Quintyn[1,2], Xin Ma[1,2], Shai Dagan[2]

[1]Ohio State University, Columbus, OH, USA, [2]University of Arizona, Tucson, AZ, USA

Keywords:
surface-induced dissociation ; collision-induced dissociation ; protein complex ; ion mobility

Novel aspects:
Surface-induced dissociation is an effective activation method for large non-covalent macromolecular assemblies, sampling well the original structure of the complex and causing less unfolding than collision-induced dissociation.

Abstract:
Surface-induced dissociation is an activation method in tandem mass spectrometry that involves collisions of ions with a surface target to induce their dissociation. This activiation method is especially useful for the dissociation of large macromolecular complexes because it provides more extensive fragmentation that collision-induced dissociation (CID) , presumably because of the large mass of the target relative the size of the complex. Examples will be presented that illustrate the surface-induced dissociation of refractory systems such as phosphorylase B that do not dissociate well by CID. SID also samples and respresents the original structure of the complex better than CID, with results reflecting whether a complex has undergone source unfolding or whether the use of different solution additives have changed the maximum charge state and structure of a complex. In CID, unfolding is common whether the activation is carried out in source or in a typical collision cell. Results for both homooligomeric (GroEL, phosphorylase B, small heat shock proteins) and heterooligomeric (toycamin nitrile hydratase, BrnT/BrnA toxin-antitoxin, tryptophan synthase) complexes will be presented. In all cases, surface induced dissociation better reflects the original structure of the complex. Both the precursor ion and the product ions remain more compact after SID than after CID, as measured by coupling ion mobility with surface-induced dissociation.

Oral Session

Tuesday, 18th September

Room D

09:00 – 11:00

Session 14: Ion-surface Collisions: Collision-induced Dissociation and Soft Landing

Chair: Jean H Futrell (Pacific Northwest National Laboratory, USA)

S14-1000

10:00 – 10:20

Mimicking redox protein function by soft landing Co(Salen) ions on self-assembled monolayer surfaces

Wen-Ping Peng[1], Grant Johnson[2], Julia Laskin[2]

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Keywords:
Co (Salen) , redox reaction, self assembly monolayer, secondary ion mass spectrometry

Novel aspects:
Charge neutralization andredox properties of Co (salen) complexes are demonstrated on self-assembledmonolayer (SAM) surfaces prepared by soft landing of mass selectedions

Abstract:

INTRODUCTION

Transition metal ions are the active sites in many naturally occurring oxygen carrier and storage proteins and, therefore, play an important role in regulating the redox balance and reactive oxygen needed for proper signaling. Co (salen) (salen = N,N'-dis (salicylidene) ethylenediamino ligand) is a common "model system " used to understand how proteins (e.g. myoglobin) function in redox signaling. Herein, soft landing of mass-selected ions is employed to achieve immobilization of catalytically active Co (salen) metal complexes on distinct self-assembled monolayer (SAM) surfaces. Using *in-situ* secondary ion mass spectrometry we demonstrate that the relative abundance of protonated Co (salen) H^+ compared to the native ion Co (salen)$^+$ following soft landing onto surfaces increases in the order FSAM <COOH-SAM< HSAM. In addition, we investigated the redox behavior of soft-landed Co (salen) complexes on the different SAM surfaces. The results indicate that oxidation of Co (salen)$^+$ occurs on the FSAM and COOH-SAM surfaces and but not on the HSAM surface, possibly due to neutralization of Co (salen)$^+$ to Co (salen) .

METHOD

To investigate the immobilization and reactivity of these model protein active sites, monomers and multimers of protonated Cobalt Salen [Co (salen) +H]$_{1\text{-}3}^+$ were soft landed onto the three distinct self-assembled monolayer (SAM) surfaces consisting of fluorinated alkanethiol (FSAM) , alkanethiol (HSAM) and hydrophilic carboxyl-terminated alkanethiol (COOH-SAM) on gold. The *in-situ* time-of-flight secondary ion mass spectrometry (TOF-SIMS) technique was employed to examine the surface composition before and after oxygen uptake by [Co (salen) +H]$^+$ on three SAMs surfaces. The surfaces were exposed to molecular oxygen in a vacuum environment for 5 minutes and subsequently reanalyzed using TOF-SIMS to monitor the redox reactivity of the immobilized Co (salen) complexes and the influence of the self assembled monolayer on the observed behavior.

PRELIMINARY DATA

Ions were generated by electrospray ionization and guided to a mass filter where species with *m/z* values corresponding to the desired size and composition (monomer, dimer, trimer) were selected and gently deposited onto three distinct SAM surfaces of FSAM, HSAM and COOH-SAM coated on gold. In the first experiment, protonated monomers of Co (salen) were soft landed on the surfaces. Then the treated surface was examined using *in situ* SIMS before and after exposure to molecular oxygen. The same procedure was employed for the dimer and trimer of [Co (Salen) +H]$_{2\text{-}3}^+$ species in a second and third experiment on same type of surfaces, respectively. SIMS spectra reveal that the relative abundance of protonated Co (salen) H^+ compared to Co (salen)$^+$ following soft landing increases in the order FSAM <COOH-SAM< HSAM. Thisv suggest that the positive charge of the Co (salen)$^+$ ion is retained on the FSAM surface. In contrast, on the HSAM surface, the Co (salen)$^+$ is rapidly neutralized following soft landing and is reionized through protonation during the SIMS analysis. The COOHSAM surface exhibits peaks consistent with both charge retention and partial protonation of some neutralized ions. As further evidence of this charge neutralization, for Co (salen) , oxidation is observed following O_2 exposure on both the FSAM and COOHSAM surface with the reaction being most pronounced on the FSAM. In contrast, no oxidation is observed on the HSAM surface. This indicates that neutralization of Co (salen)$^+$ and reduction of the Co oxidation state reduces oxidation reactivity with O_2. Comparing the isotope ratios for the dimer of Co (salen)$^+$ on FSAM, COOHSAM, and HSAM shows that on FSAM and COOHSAM the dominant ion is [Co (salen)]$_2H^+$ at *m/z* = 651. Comparing the isotope ratios for the trimer of Co (salen)$^+$ on FSAM, COOHSAM, and HSAM shows that on FSAM and COOHSAM the dominant ion is [Co (salen)]$_3H^+$ m/z= 976 and [Co (salen)]$_3H_2^+$ m/z = 977,respectively.

Oral Session

Tuesday, 18th September

Room D

09:00 – 11:00

Session 14: Ion-surface Collisions: Collision-induced Dissociation and Soft Landing

Chair: Jean H Futrell (Pacific Northwest National Laboratory, USA)

S14-1020

10:20 – 10:40

Epitaxy and Nanostructure Growth by Electrospray Ion Beam Deposition: Nonvolatile Molecules, Peptides, Proteins

Stephan Rauschenbach[1], Gordon Rinke[1], Matthias Pauly[1], Ludger Harnau[2,3], Klaus Kern[1,4]

[1]Max-Planck-Institute for Solid State Research, Stuttgart, Germany, [2]Max-Planck-Institute for Intelligente Systeme, Stuttgart, Germany, [3]Institute for Theoretical und Applied Physics, Univ. Stuttgart, Germany, [4]Institut de Physique de la Matiere Condensee, Ecole Polytechnique Federale de Lausanne, Switzerland

Keywords:
soft landing, scanning tunneling microscopy, electrospray ion beam deposition, protein, peptide

Novel aspects:
We show that soft landing/ion beam deposition can indeed be used equivalent to molecular beam epitaxy. It is further shown, that for large molecules the growth kinetics becomes very important.

Abstract:
Ordering phenomena can lead to materials with unique and often very useful properties. For instance, a long-range ordered piece of silicon is a semiconductor, perfectly suited for electronic devices. Another example, an ordered-which means folded-polypeptide chain is a functional protein. Clearly, the control of ordering is of pivotal importance in material science.

Traditionally, molecular beam epitaxy (MBE) is the vacuum deposition approach to fabricate highly ordered thin films and self-organized nanostructures. Atoms and molecules are evaporated and the vapor is condensed on a surface in ultrahigh vacuum (UHV, 10^{-10} mbar) to avoid contamination. Here we show that for nonvolatile substances like large organic molecules, peptides or proteins, electrospray ion beam deposition (ES-IBD) can serve as an equivalent vacuum deposition method to produce highly ordered coatings and nanostructures. In ES-IBD intact molecular gas phase ions, generated by electrospray ionization (ESI) , are conveyed to a surface in UHV, using ion optics within a differentially pumped vacuum system.

Despite the similarities, the differences between MBE and ES-IBDare significant. Specifically in MBE neutral particles are deposited with thermal energies, in ES-IBD the particles are charged and have hyperthermal energies. The advantage of using charged particles instead of neutrals is that this offers a maximum level of process control, specifically by mass spectrometry, mass- and charge state selection, energy selection and quantitative coverage monitoring. The deposition energy of the hyperthermal particles however, often exceeds the interaction energies that govern the ordering phenomena at the surface.

Using scanning tunneling microscopy in ultrahigh vacuum, we show for several small nonvolatile molecules that ES-IBD can be used to create crystalline coatings at the surface that can be extended into the third dimension.[1]For larger molecules, specifically peptides, we show ordering of the internal degrees of freedom at the surface after ES-IBD. They are found to self-assemble into one preferred configuration, while other configurations appear less abundant. Another step up in size from peptides, unfolded proteins deposited to a surface are not found to reach a preferred, two-dimensional equilibrium structure at the surface.[2]

[1] S. Rauschenbach, G. Rinke, N.Malinowski,T. Weitz, N. Thontasen, Z. Deng, T. Lutz, T. P. M. de Almeida Rollo,G. Costantini, L. Harnau, K. Kern : *Crystalline Inverted Membranes Grown on Surfaces by Electrospray Ion Beam Deposition inVacuum.* ***Adv.Mater.*** *(*2012)

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Oral Session

Tuesday, 18th September

Room D

09:00 – 11:00

Session 14: Ion-surface Collisions: Collision-induced Dissociation and Soft Landing

Chair: Jean H Futrell (Pacific Northwest National Laboratory, USA)

S14-1040
10:40 – 11:00

Soft and Reactive Landing of Ions for the Fabrication of Bioactive Metal Surfaces

Michael Volny, Joelle M Rolfs, Tim Elam, Frantisek Turecek
University of Washington, Seattle, USA

Keywords:
soft landing, reactive landing, sensors, surface modification

Novel aspects:
application of reactive landing for unique surface modification and for development of sensors

Abstract:
Ion soft landing (SL) and reactive landing (RL) are techniques in which hyperthermal polyatomic ions are nondestructively deposited onto a surface under controlled conditions of kinetic energy, surface composition, roughness, etc. RL refers to non-destructive immobilization of (bio) molecular ions on the surface following hyperthermal impact during SL. Conceived originally as a physical method for deposition of atomic ions and later applied to fundamental studies of gas-phase ions SL studies have been extended to achieve various goals.
SL and RL were used to deposit proteins, large organic molecules, or to modify self-assembled monolayers. Previous research has established SL as a method for multiplex separation of biomolecules and demonstrated that biomolecular ions soft-landed on dry surfaces have been shown to retain their original molecular structure and/or bioactivity. The reactively landed species adhere to the surface without losing their specific properties (enzymatic activity, molecular recognition, etc.) even after prolonged washing by solvents, buffers, and detergents. RL is a special type of a surface modification by molecular ions that, as experimentally demonstrated, most likely represents a unique transition between physisorption and chemisorption phenomena on metal/metaloxide surfaces. Although the amount of material that can be processed by SL and RL is practically limited to high picomoles or low nanomoles, the continuing miniaturization of scientific instruments and overall downscaling of experiments in molecular sciences make soft landing perfectly compatible with current methods of life-science research.

Several research groups have developed methods to characterize the soft and reactively landed molecules that included specific enzyme and binding assays, fluorescence imaging, secondary ion mass spectrometry, IR spectroscopy and surface enhanced Raman scattering (SERS) spectroscopy. SERS was shown to allow detection of soft-landed material at sub-attomole levels.

This presentation will be focused on application of RL for unique surface modifications that are difficult or impossible to be achieved by other means. Fabrication process based on reactive landing of ions has been developed to produce surfaces with specific activity. For instance, hyaluronic acid (MW 420,000) , a polysaccharide that prevents protein adhesion to surfaces and activation of blood platelets by foreign bodies, was reactively landed on plasma-treated 316 stainless steel and shown to prevent blood platelet activation when exposed to whole blood during platelet adhesion studies. Stainless steel and other conductive surfaces were also modified by reactive landing of titanium and zirconium propoxide for selective enrichment of phosphopeptides from complex peptide mixtures. The latest application of RL is for surface coating of metal and semiconductor materials to fabricate biosensors based on conductivity measurements.

Oral Session

Tuesday, 18th September

Room E

09:00 – 11:00

Session 15: Mass Spectrometry for Nuclear Applications and Safety

Chair: Nobuo Takaoka (Kyushu Univertsity, Japan)

S15-0900 [Keynote Lecture] Extractive Electrospray Ionization Mass Spectrometry for Uranium Chemistry Studies

09:00 – 09:40

Huanwen Chen, Mingbiao Luo, Saijin Xiao, Yongzhong Ouyang, Yafei Zhou, Xinglei Zhang
East China Institute of Technology, Nanchang, P.R. China

Keywords:
EESI, uranium, chemistry, rapid detection

Novel aspects:
Progress of EESI-MS on uranium chemistry including detection, synthesis and characterization will be presented.

Abstract:
Uranium chemistry is of sustainable interest. Breakthroughs in uranium studies make serious impacts in many fields including chemistry, physics, energy and biology, because uranium plays fundamentally important roles in these fields. Substantial progress in uranium studies normally requires development of novel analytical tools.
Extractive electrospray ionization mass spectrometry (EESI-MS) is a sensitive technique for trace detection of various analytes in complex matrices without sample pretreatment[1-5]. EESI-MS shows excellent performance for monitoring uranium species in various samples at trace levels since it tolerates extremely complex matrices. Therefore, EESI-MS is an alternative choice for studying uranium chemistry, especially when it combines ion trap mass spectrometry.
In this presentation, examples of EESI-MS for uranium chemistry studies will be given, illustrating the potential applications of EESI-MS in synthesis chemistry, physical chemistry, and analytical chemistry of uranium. More specifically, case studies on EESI-MS for synthesis and characterization of novel uranium species, and for rapid detection of uranium[4] and its isotope ratios[5] in various samples will be presented. Novel methods based on EESI-MS for screening uranium ores and radioactive iodine-129 will be presented.

Acknowledgement(s)
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Oral Session

Tuesday, 18th September

Room E

09:00 – 11:00

Session 15: Mass Spectrometry for Nuclear Applications and Safety

Chair: Nobuo Takaoka (Kyushu Univertsity, Japan)

S15-0940

09:40 – 10:00

Importance of process blank in isotope ratio determination of uranium and plutonium in ultra trace levels

Sunyoung Lee, Young Geun Ha, Jong-Ho Park, Kyuseok Song

Korea atomic energy research institute, Daejeon, Korea

Keywords:
MC-ICP-MS, Uranium, Plutonium, Nuclear safeguard, Isotopic analysis

Novel aspects:
We demonstrated that U and Pu can be separated from the isotopes blend and quantitative (few ng of U and pg of Pu) and isotopic analyses were performed by MC-ICP-MS.

Abstract:
Highly accurate and precise analysis for determining isotope ratios of uranium (U) and plutonium (Pu) plays a key role in monitoring undeclared nuclear activities for safeguards and nuclear forensics. Multi-collector inductively coupled plasma mass spectrometery (MC-ICP-MS) which allows simultaneous measurements for the isotopes of one or more elements has emerged as a powerful analytical tool to determine U and Pu isotopes in ultra-trace levels. Prior to the mass spectrometric analysis, an additional chemical separation is required to separate individual elements. However, we observed variation of process blank derived from the chemical separation. Several factors such as lab environment, separation methods, and chemical equipment may influence on the process blank that becomes a critical issue for the accurate isotopic ratio determination in ultra trace levels. The separation process followed by an acid treatment provides pico-gram level of process background of U. Finally, we demonstrated that U and Pu can be separated from the isotopes blend and quantitative (few ng of U and pg of Pu) and isotopic analyses were successfully performed by MC-ICP-MS.

Oral Session

Tuesday, 18th September

Room E

09:00 – 11:00

Session 15: Mass Spectrometry for Nuclear Applications and Safety

Chair: Nobuo Takaoka (Kyushu Univertsity, Japan)

S15-1000

10:00 – 10:20

Fissiogenic noble gases anomaly associated with Fukushima-daiichi nuclear power plant disaster after the 3.11 Northeast Japan Earthquake

Keiko Sato[1], Hidenori Kumagai[1], Naoyoshi Iwata[2], Hironobu Hyodo[3], Katsuhiko Suzuki[1]

[1]IFREE, JAMSTEC, Yokosuka, Japan, [2]Faculty of Science,Yamagata University, Yamagata, Japan, [3]RINS, Okayama University of Science, Okayama, JAPAN

Keywords:
noble gas, fissiogenic, 3.11 Northeast Japan Earthquake, nuclear power plant disaster

Novel aspects:
In our series of sampled atmosphere covering over Japanese islands, relative elemental abundances and isotope ratio in heavier noble gases were changed ; Xenon and Krypton were enriched to pre-disaster atmosphere.

Abstract:
Noble gases have unique characteristics that they are rarely combined with other chemicals as their very stable nature. Because its main reservoir is atmosphere, their isotopic composition is well defined and believed to be uniform all over the world insensitive to disturbance from anthropogenic and/or natural emission of geologically trapped noble gases in the earth interior. Based on our preliminary friction experiment, however, detectable amount of noble gases seem to be emitted accompanied with a fault motion (Sato et al., 2009) . After the extreme Northeast Japan Earthquake occurred on March 11, 2011, extraordinary increase of seismic activity as numerous aftershocks e.g. over 4000 felt earthquakes in four months, which may be a source of non-atmospheric component preserved in the earth interior.

In terms of anthropogenic component, NPP (Nuclear Power Plant) is a potential source, which is frequently monitored by radioactive species, e.g. Xe-133, Xe-135. A serious NPP accident has occurred due to 3.11 Northeast Japan Earthquake and accompanying extreme tsunami.

We widely collected atmosphere samples all over Japan : two from Hokkaido Island (Sapporo and Akan) , eight from Tohoku and Kanto District (Sendai, Yamagata, Koriyama, Miharu, Tsukuba, Akiruno, Chiba and Yokosuka) , three from Southeast Japan (Osaka, Okayama and Nangoku) , two from Kyushu Island (Beppu and Aso) . The atmospheres were sampled into vacuumed containers, Isotube@, at each sampling site. Further sampling to evaluate time-series change still continues.

The elemental and isotopic compositions of the samples were analyzed mainly by quadrupole residual gas analyzers (RGA-200, SRS Co.) and partly confirmed by sector-type mass spectrometers (GVI-5400, GV instruments) . In the duplicated analyses of the selected a few samples, the measured elemental and isotopic compositions were consistent within analytical uncertainties.

In our series of sampled atmosphere covering over Japanese islands, from Kyushu to Hokkaido, relative elemental abundances in heavier noble gases were changed ; Xenon and Krypton were enriched to pre-disaster atmosphere. Further, radioactive and fissiogenic Xenon and Krypton isotopes are significantly higher than those in **"pre "** 3.11 Earthquake atmospheres. It includes unstable isotopes with short half-lives (e.g. Kr-85, Xe-133, Xe-135) and stable (daughter) isotopes (e.g. Kr-86, Xe-134, Xe-136) , which suggests by the nuclear plant disaster as their source. Thus, the atmospheric xenon isotopic composition in Eastern Japan area until last typhoon season is similar to those reported as in the geological samples taken from the vicinity of Natural Nuclear Reactor in the Oklo (e.g. Kuroda, 1992 ; Meshik et al., 2004) and Okelobonde (e.g. Meshik et al., 2000 ; Meshik et al., 2004) . Additionally, Ar-41, Ar-42, Ar-39 are also detected in the sampled atmosphere as observed in neutron irradiated geological samples in Ar-Ar dating.

Oral Session

Tuesday, 18th September

Room E

09:00 – 11:00

Session 15: Mass Spectrometry for Nuclear Applications and Safety

Chair: Nobuo Takaoka (Kyushu Univertsity, Japan)

S15-1020
10:20 – 10:40

Investigations of Different Sample Matrices in Uranium Abundance Analysis Using the MTE Methodology

Claudie K Black, Evelyn Zuleger, Joan Horta-Domenech, Martin Vargas Zuniga
Institute for Transuranium Elements, Joint Research Centre, European Commission, Karlsruhe, Germany

Keywords:
TIMS, Nuclear, Safeguards, Uranium, Plutonium

Novel aspects:
Insight into differing run parameters and preparations for different sample matrices.

Abstract:
The ITU in Karlsruhe routinely analyses a multitude of samples from a wide range of internal and external customers. High throughput analysis techniques are employed using meticulous care to ensure accurate, precise and timely results are provided. Whilst the samples are generally the same there is a diversity of sample matrices, each of which requires individual chemical processing for analysis. This can give rise to differences in analytical run performance. TIMS is used for isotopic analysis of Uranium and Plutonium to determine abundance and concentration information. More recently there has been an increase in requests for Uranium minor isotope analysis for the U234 and U236 isotopes. At ITU we employ the MTE methodology [1] for minor isotope analysis and have observed varying run quality between samples of differing matrix. This presentation will provide some insight into the investigations carried out into the differences observed and how they can be improved for consistent quality routine analysis. [1] S. Richter et al, J. Anal. At. Spectrom., 2011, 26, 550564

S15-1040
10:40 – 11:00

Selective analysis of individual uranium particles with higher U-235 abundance by secondary ion mass spectrometry

Fumitaka Esaka, Chi-Gyu Lee, Masaaki Magara, Takaumi Kimura

Japan Atomic Energy Agency, Ibaraki, Japan

Keywords:
SIMS ; Uranium ; Particles ; Fission-track

Novel aspects:
Analytical techniques to select and measure individual uranium particles with higher U-235 abundance in environmental samples have been developed.

Abstract:
Uranium isotopic signature in dust samples taken at nuclear facilities gives important information on nuclear activities. The presence of undeclared nuclear activities related to the production of nuclear weapons can be deduced by analyzing isotope ratios of individual uranium particles. In particular, the detection of uranium particles with higher ^{235}U abundance has a crucial role to unveil undeclared nuclear activities. Secondary ion mass spectrometry (SIMS) is commonly used for measuring uranium isotope ratios in individual particles [1,2] . Since uranium particles in the sample are randomly measured with SIMS, it is difficult to detect a small number of uranium particles with higher ^{235}U abundance in a large number of natural and depleted uranium particles. In this work, we have studied on the SIMS technique combined with pre-screening of uranium particles by a fission track (FT) technique [3] or an automated particle measurement (APM) to analyze uranium particles with higher ^{235}U abundance selectively.
Uranium standard materials (NBL CRM U950a and U100) and inspection swipe samples taken at nuclear facilities were used in this work. Particles in the sample were recovered onto a glassy-carbon planchet for APM-SIMS analysis, which allows to perform pre-screening of isotopic composition for individual particles [4] . For the FT technique, particles in the sample were recovered onto polycarbonate membrane filters. The filters were dissolved to make polycarbonate films containing particles. The films were then irradiated with thermal neutrons in a nuclear research reactor. As the result, fission tracks were created and observed with an optical microscope. The uranium particles having a large number of fission tracks were selected for subsequent analysis, because the number of fission tracks depends on the amount of ^{235}U in the particle. Finally, the uranium isotope ratios of the selected particles were measured with SIMS.
In the analysis of an inspection sample with APM-SIMS, we could detect uranium particles by obtaining ^{235}U and ^{238}U ion images. The uranium particles with higher ^{235}U abundance ratio were then selected and the isotope ratios were measured by SIMS with micro-beam to obtain precise isotope ratios. In the FT-SIMS analysis for an inspection sample, we measured 20 particles having the ^{235}U abundance ratio between 2.8 and 4.6. The range was higher than that measured by SIMS without using the FT technique. In conclusion, both methods examined in this work are effective to identify and analyze uranium particles with higher ^{235}U abundance.
This work was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Oral Session

Tuesday, 18th September

Main Hall

15:00 – 17:00

Session 16: Glycoanalytical Technology for Systems Glycobiology and Functional Glycomics

Chair: Jane Thomas-Oates (University of York, UK)

S16-1500

15:00 – 15:40

[Keynote Lecture] Glycoanalytical technologies for systems glycobiology, biomakrer discovery & therapies

Radka Fahey, Eugune Dempsey, Karna Marino, Amanda McCann, Pauline M Rudd
NIBRT - Ireland

Keywords:
Systems Glycobiology, Glycan analyics, Biomarkers, Genomics/Glycomics

Novel aspects:
First demenstration of carmelations between gemone and glycome GWAS study of more than 75,000 people using Hight Theraputic glycoanalysis

Abstract:
Glycoanalytical technologies for systems glycobiology, biomarker discovery and therapies
Pauline M Rudd. Glycobiology Group, National Institute for Bioprocessing Research and Training, Fosters Avenue, Mount Merrion, Blackrock, Co.Dublin, Ireland

Over half of all proteins are glycosylated, and alterations in glycosylation are common in physiological and pathological processes. Glycan structures are, in the first instance, controlled by genes, however the complex pathways and epigenetic factors that regulate their processing provide a further mechanism for fine tuning and diversifying the glycans and the functions of the proteins to which they are attached. Thus in the determining changes of glycosylation in disease it is imperative to understand the systems which are perturbed to generate the altered glycan processing. To understand the relationship between the glycome and other omics data as well as to identify and validate potential glycosylated biomarkers a robotic platform capable of releasing and labelling glycoproteins in a 96 well plate format has been developed as a front end to various glycan separations technologies including HILC and RP HPLC, MS and capillary electrophoresis, chip technologies and on-line combinations of these techniques. Data bases for all of these technologies are currently under construction (http：//glycobase.nibrt.ie/tools.html). This platform allows the glycan structures to be profiled, characterised or analysed in detail. By combining genome-wide association studies and high-throughput glycomics analysis of 3000 individuals we identified SNPs in genes encoding Hepatocyte Nuclear Factor 1 α (HNF 1 α) and fucosyltransferases FUT 6 and FUT 8 that modulate the fucosylation of human plasma glycoproteins, including IgG. HNF 1 α and its downstream target HNF 4 α are both necessary and sufficient to regulate the expression of key fucosyltransferase and fucose biosynthesis genes in hepatic cells. Interestingly, levels of outer arm fucosylated glycans proved to be an effective marker for Mid-Onset Diabetes of the Young (MODY). Now that it is possible to link different -omics data together in a systems approach to Glycobiology, new insights are emerging that suggest new control points for glycosylation, relevant to clinical marker discovery.

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Oral Session

Tuesday, 18th September

Main Hall

15:00 – 17:00

Session 16: Glycoanalytical Technology for Systems Glycobiology and Functional Glycomics

Chair: Jane Thomas-Oates (University of York, UK)

S16-1540

15:40 – 16:00

A Rapid-Throughput Platform for Quantitation of Site-Specific Glycosylation with Differentiation of Structural Isomers

Serenus Hua[1], Chloe Hu[2], Charles C Nwosu[2], John S Strum[2], Carlito B Lebrilla[2], Hyun Joo An[1]

[1]Chungnam National University, Daejeon, South Korea, [2]University of California, Davis, USA

Keywords:
site-specific, glycoproteomic, quantitation, biomarkers, biotherapeutics

Novel aspects:
Automated workflow for rapid yet highly reproducible quantitation of site-specific glycosylation

Abstract:
The type, extent, and location of glycosyl modifications on protein therapeutics mediates their *in vivo* activity, their rate of clearance from the body, and ultimately, their effectiveness. However, few methods are available to simultaneously monitor all of these characteristics. We have developed a highly reproducible method of rapidly characterizing and quantifying both *N*- and *O*-linked glycosylation within the context of their location within a protein or protein mixture. Our method provides comprehensive analysis of glycan structure, glycosylation site, and relative quantities of all significant glycoforms in a system.

Glycoproteins are rapidly digested in a microwave reactor using controlled broad-specificity proteolysis in order to produce informative glycopeptides. Due to the effects of microwave energy, our method is very quick, with digestion times measured in mere minutes rather than the hours or days required for conventional protease digests.

Following enrichment by graphitized carbon or hydrophilic interaction (HILIC) solid-phase extraction, glycopeptides are separated and analyzed by chip-based porous graphitized carbon (PGC) nano-LC/MS and nano-LC/MS/MS. Automated data extraction, processing, and interpretation facilitates site-specific characterization and quantitation of the glycoprotein and associated glycoforms. The rapid-throughput workflow takes, on average, about 40 minutes per sample (36 samples per day) including sample digestion, clean-up, and nano-LC separation.

In general, chip-based PGC nano-LC/MS identified hundreds of chromatographic peaks per glycopeptide injection. Of these, over 100 glycopeptide peaks per injection could be confirmed and identified by MS/MS (limited mainly by the per-spectrum acquisition time of the mass spectrometer). Combined with the structure-sensitive properties of PGC, chip-based nano-LC enabled baseline separation of pairs, triplets, and quadruplets of glycopeptide structural isomers. Structural differences between isomers were characterized by accurate-mass MS/MS.

The method was found to be extremely sensitive. Glycopeptides were detected and successfully profiled from as little as 10 nanograms (or approximately 600 femtomoles) of starting glycoprotein. Dynamic range spanned five orders of magnitude, so glycopeptides from low-occupancy glycosylation sites could be detected alongside those from higher-occupancy sites. Using this method, up to 12 glycosylation sites have been simultaneously monitored for glycosylation thus far.

The abundance and species of glycopeptides generated by this method were found to be highly reproducible, given the same digestion conditions. Calibration curves were successfully fit to digestions of various concentrations, enabling accurate quantitative analysis of site-specific glycosylation.

Several *N*- and *O*- glycosylated proteins have been digested and analyzed using this method, including prostate-specific antigen (a cancer biomarker), recombinant erythropoietin (EPO), and several therapeutic monoclonal antibodies (mAbs). Protein mixtures have also been analyzed successfully. Combined with the automated data extraction and spectral interpretation software package recently developed by our lab, this method can be used to rapidly and quantitatively profile large glycosylated sample sets, with clear applications to quality control of therapeutic glycoproteins; drug testing and screening; glycoproteomic biomarker discovery; and a host of other functions.

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Tuesday, 18th September

Main Hall

15:00 – 17:00

Session 16: Glycoanalytical Technology for Systems Glycobiology and Functional Glycomics

Chair: Jane Thomas-Oates (University of York, UK)

S16-1600 Glycome Signature for Cancer Cell Identification

16:00 – 16:20

Hyun joo An[1], Lauren Dimapasoc[2], Mary Saunders[2], Seunghyup Jeong[1], Jaehan Kim[1], Kit Lam[2], Carlito B Lebrilla[2]

[1]ChungNam National University, [2]University of California, Davis

Keywords:
cell surface, glycosylation, mass spectometry, glycan mapping

Novel aspects:
Comprehensive characterization of cancer cell membrane glycomes performed for the first time by mass spectrometry

Abstract:
Every cell in an organism synthesizes a heterogeneous array of glycans in the form of various structures. Cell surface glycosylation may play an important role in development and may provide important new sources of markers for differentiation. However, studies regarding the glycosylation of cell surfaces are limited due to the lack of sensitive analytical methods. We have recently developed a method to measure cell surface glycosylation and provide extensive characterization of the heterogeneity of the human embryonic stem cells (hESC) membrane proteins. Enrichment of membrane proteins followed by high performance mass spectrometry and chromatography makes this approach both highly specific and sensitive. The resulting approach provides comprehensive and highly quantitative structural information including isomer separation.
Using this new method, we have profiled surface glycans from more than 40 cancer cell lines. Each cancer cell displayed not only common glycans of cancer cells but also unique glycans that have the characteristic features of specific cell types. Interestingly we discovered that most of the cancer cells such as breast cancer (MCF 7-her2, MCF 7-C6, MCF 7-WT, ZR751), ovarian cancer (SKOV-3 and ES-2), intestine cancer (HT-29 and Caco-2), leukemia (Jurkat, TK6, molt 4), prostate cancer (PC 3), and cervical cancer (CCL 2) have high levels of high mannose glycans on the cell surface. These findings suggest that high-mannose glycans are the major component of cancer cell surface glycosylation. The amount of high mannose provide indications that misglycosylation in cancer occurs mainly in the endoplasmic reticulum. High mannose glycans are not commonly presented on the surfaces of normal mammalian cells or in human serum, yet they may play important roles in cancer cell biology. Hierarchical cluster and PCA analysis of human cancer cell lines were also performed to examine the correlation of cancer cells based on glycan profile.
The results represent that glycomic signatures on cell membrane may be used for the identification of specific cancer cells. The results also mean that distinguishing cancer cells from other mammalian cells may be facilitated by the major difference in the glycosylation of the cell membrane.

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Main Hall

15:00 – 17:00

Session 16: Glycoanalytical Technology for Systems Glycobiology and Functional Glycomics

Chair: Jane Thomas-Oates (University of York, UK)

S16-1620

16:20 – 16:40

Enhancement of carbohydrate signal by diamond nanoparticles in MALDI-MS

Chieh-Lin Wu[1], Chia-Chen Wang[2], Yin-Hung Lai[1], Yuan Tseh Lee[1], Yi-Sheng Wang[1,2]

[1]Academia Sinica, [2]National Yang-Ming University and Academia Sinica

Keywords:
nanodiamond, carbohydrate, MALDI-MS, triple-layer

Novel aspects:
Incorporation of diamond nanoparticles in MALDI sample enhances the ion yield of carbohydrates. The method allows the detection of carbohydrates when they are mixed with comparable amount of proteins.

Abstract:
Enhancements of carbohydrate signal by incorporating nanodiamond (ND) into the samples of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) are demonstrated. In this work, we used ND as an additive of 2,5-dihydroxybenzoic acid (2,5-DHB) and developed a new sample preparation method. Our new approaches revealed that ND improves carbohydrate signal and enhanced signal-to-noise ratio (S/N) by decreasing noise.

The ND was selected as the additive in this study because it is chemically inert and it possesses many advantages over other nanoparticles. First, ND is transparent in the UV-A region, which is the most employed laser wavelength range in MALDI-MS. Therefore, the presence of ND in matrix crystals does not attenuate the laser energy for matrix ionization. Furthermore, because ND is an electrically insulator and is chemically inert, it does not react with matrices or analytes under the high temperature condition of MALDI. Other metallic nanoparticles may induce side reactions under a harsh ionization environment. The superior thermal conductivity of diamond may also contribute to the enhancement of ion yield of thermal labile molecules. A detail discussion of the method will be presented.

The best S/N was obtained using a triple-layer preparation method, in which the sample was prepared and vacuum-dried successively with the matrix in the bottom, ND in the middle and analyte in the upper layer. The S/N of carbohydrates was increased with increasing the particle size of ND. In comparison with non-ND samples, the use of ND increased the S/N of dextran (1.5 KDa) and β-cyclodextrin by an order of magnitude and more than fifty times, respectively. For a large polysaccharide, pullulan (9.6 KDa), higher signal intensity than non-ND samples was also observed. The method also facilitated the detection of dextran that was mixed with equal amount of insulin B chain. Systematic comparisons between the data obtained with various sample conditions are made to study the reaction mechanism, including the removal of ND, the size, amount and distribution of ND. The morphology of the triple-layer sample was studied using fluorescence lifetime imaging microscopy (FLIM). In conclusion, the new sample preparation method improves the detection efficiency of carbohydrates and it can be further applied to the glycomic studies.

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Tuesday, 18th September

Main Hall

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Session 16: Glycoanalytical Technology for Systems Glycobiology and Functional Glycomics

Chair: Jane Thomas-Oates (University of York, UK)

S16-1640

16:40 – 17:00

Electron-based fragmentation approaches for glycans and glycoconjugates

Catherine E Costello, Joseph Zaia, Cheng Lin
Boston University School of Medicine

Keywords:
glycans, tandem mass spectrometry, cross-ring fragments, ExD, glycoconjugates

Novel aspects:
Increased fragmentation for glycans enables rapid, efficient and highly sensitive structural assignments

Abstract:
For the full structural determination of glycan structures, glycosidic fragmentation that defines sequence and cross-ring fragmentation that specifies linkage positions are both required. Under low-energy collision and IRMPD conditions, glycosidic fragments dominate the spectra. We are exploring various electron-based fragmentation approaches (ExD) and have established conditions that meet both criteria, usually requiring only MS^2 dissociations, with data acquisition rates that are compatible with LCMS, even for high-performance FTMS experiments. These spectra are information-rich and can be utilized for automated interpretation. Development of the techniques and application to structural problems will be discussed.
This research is supported by NIH Grants P41 RR10888/GM104603, S10 RR020946 and S10 RR025082 and NIH-NHLBI contract N01 HV00239.

Oral Session

Tuesday, 18th September

Room A

15:00 – 17:00

Session 17: Non-Covalent Ion-Molecule Interactions

Chair: Seung-Koo Shin (Pohang University of Schence and Technology, Korea)

S17-1500 [Keynote Lecture] The Thermochemistry of Non-Covalent Ion-Molecule

15:00 – 15:40

Peter B Armentrout

University of Utah, Salt Lake City, USA

Keywords:
Molecular recognition, Collision-induced dissociation, Bond-energies

Novel aspects:
Thermodynamic information about a wide variety of non-covalent complexes is obtained and reveals the details controlling the strength of these interactions.

Abstract:
The use of mass spectrometry to study non-covalent interactions has a long history and pertains to phenomena ranging from ion solvation to host-guest complexes to protein folding. In all of these cases, electrostatic interactions often dominate the formation of the complexes of interest but more subtle interactions are also at play, changing the probabilities that Coulomb fission processes can occur, that host-guest complexes have selective interactions useful in environmental remediation, and that protein folding is directed and not stochastic.

Energy-resolved collision-induced dissociation in a guided ion beam tandem mass spectrometer is a method developed over the past years that permits the measurement of the energetics for a variety of non-covalent interactions. Experiments involve measuring the probability for dissociation of an appropriate complex induced by collisions with an inert gas as a function of the kinetic energy of the ion, which can be varied over a wide range. Analysis of such absolute cross section data, taking into account multiple collisions, the lifetimes for dissociation, and the energy distributions of the reagents, allows the extraction of the intrinsic affinity between the ion and various ligands, both simple and complex. Such absolute data can often be compared with quantum chemical reactions, thereby clearly identifying the structures of the reactant complexes and the mechanisms for their association/dissociation.

In this presentation, examples of such measurements as applied to metal dication hydration and metal cation complexes with amino acids and crown ethers will be examined. The trends in such data reveal some of the complexities that control the energetics of these interactions.

Oral Session

Tuesday, 18th September

Room A

15:00 – 17:00

Session 17: Non-Covalent Ion-Molecule Interactions

Chair: Seung-Koo Shin (Pohang University of Schence and Technology, Korea)

S17-1540
15:40 – 16:00

Atomic metal anions: one-step dehydrogenation and defluoronation of linear and cyclic hydrocarbons, alcohols and their fluoronated analogues

Paul M Mayer[1], Alex Mungham[1], Jeffery Butson[1], Jaleh Halvachizadeh[1], Dhiya Hassan[1], Sharon Curtis[1]
[1]University of Ottawa, Ottawa, Canada

Keywords:
metal, anions, dehydrogenation, defluorination, ligand

Novel aspects:
This work is the first extensive examination of the reactions of atomic metal anions, and demonstrates new reactions with alcohols, alkanes and fluorinated systems.

Abstract:
The generation and reactivity of gas-phase metal cations has been a focus of mass spectrometry related studies for quite some time. This has mostly been related to adduct formation and their involvement as reagents in chemical ionization processes. Our research group has recently discovered a way to generate a wide variety of metal anions (Na^-, K^-, Rb^-, Cs^-, Fe^-, Ni^-, Co^-, Cu^-, Ag^-) using a simple commercial electrospray mass spectrometer. The metal anions were all generated by the decomposition of metal cation carboxylate complexes. Owing to the previous difficulties associated with generating these unique species, the idea of reactive gas phase atomic metal anions has been relatively untouched. We will first present the results of theoretical calculations on the precursor metal-oxalate anions with a view to understanding the formation of the metal anion upon CID of the complex. The performance of a wide selection of methods was assessed and a robust computational approach to these systems was established. Clear from the calculations is that the situation for the transition metal anions is complicated by several low-lying electronic states, making the computational estimate of their properties a multi-determinant problem. None-the-less, evidence is seen for the insertion of metal atoms into the C-C bond in the oxalate dianion, a reaction that leaves the metal already primarily negatively charged. In some cases, the calculated structure is essentially a MCO_2 anion interacting with a neutral CO_2 molecule at some distance. Experimentally, this study investigates the previously unexplored reactivity of atomic metal anions produced in the source of a triple quadrupole mass spectrometer with several volatile organic molecules. One class consisted of the reaction of Fe- with the primary alcohols methanol through heptanol, secondary alcohols 2-propanol and 2butanol and the tertiary alcohol t-butanol. Dehydrogenation of the alcohols (except for methanol) was demonstrated through the observation of FeH_2^- in the resulting mass spectrum. Labelling studies showed that this dehydrogenation takes place in the reaction of Fe^- with a single alcohol molecule, and not in the two-step process previously observed for metal carbonyl anions such as $Fe(CO)_2^-$. In the case of the smaller alcohols methanol, ethanol and propanol, $FeOR^-$ and $FeOH^-$ anions are observed. Fe^- was also observed to dehydrogenate alkanes in a similar manner. Theory has been applied to chart the mechanism of the dehydrogenation reaction and the results will be presented in conjunction with rate constant measurements on an ion trap mass spectrometer. Metal anions have also been allowed to react with fluorinated ring-systems such as per-fluorophenol and hexafluorobenzene. Here there is evidence for competitive deprotonation of the phenol OH group and F^+ abstraction from the ring.

Oral Session

Tuesday, 18th September

Room A

15:00 – 17:00

Session 17: Non-Covalent Ion-Molecule Interactions

Chair: Seung-Koo Shin (Pohang University of Schence and Technology, Korea)

S17-1600

16:00 – 16:20

Structural and Energetic Effects in the Molecular Recognition of Peptides and Proteins by 18-Crown-6: Threshold Collision-Induced Dissociation and Theoretical Studies

Mary T Rodgers, Ranran Wu

Wayne State University

Keywords:

amino acids ; binding affinities ; collision-induced dissociation ; crown ethers ; molecular recognition

Novel aspects:

The first determination of the absolute 18-crown-6 binding affinities of a variety of protonated peptidomimetic bases, amino acids, N-acetylated amino acids, and side-chain acetylated amino acids.

Abstract:

The SNAPP (selective noncovalent adduct protein probing) technique relies on selective binding of 18-crown-6 (18C6) or other crown ethers to peptides and proteins to facilitate identification and characterization of protein sequence, structure, and conformational changes. Accurate structural and thermochemical information regarding the binding between 18C6 and related model systems may provide insight into the factors that lead to selectivity in the complexation process. Unfortunately, very limited structural and thermochemical data has been reported in the literature. Therefore, we use energy-resolved collision-induced dissociation (CID) measurements coupled with threshold analyses of the measured CID cross sections and density functional theory calculations to characterize the structures anad 18C6 affinities of a variety of models systems including protonated peptidomimetic bases and natural and acetylated amino acids to elucidate structural and energetic effects that lead to selectivity in the molecular recognition of peptides and proteins by 18C6. Nine peptidomimetic bases were investigated that include a series of primary amines as models for the n-terminal amino group and the side chain of lysine (Lys) residues including methylamine, ethylamine, n-propylamine, i-propylamine, n-butylamine and 1,5-diaminopentane ; imidazole and 4-methylimidazole as models for the side chain of histidine (His) ; and 1-methylguanidine (MGD) as a model for the side chain of arginine (Arg) . In addition, five amino acids including glycine (Gly) , alanine (Ala) , Lys, His, and Arg as well as the backbone acetylated forms of Lys, His and Arg, and the side-chain acetylated form of Lys, were also investigated. Collision-induced dissociation of these complexes results in loss of intact 18C6 in all systems allowing the absolute 18C6 affinities of these species to be determined. In addition, loss of the intact base or amino acid is observed in competition with loss of 18C6 for many systems, allowing the absolute base or amino acid affinities of protonated 18C6 to be determined. Competitive analyses of these latter systems also allow the relative proton affinities (PAs) of 18C6 and the associated base or amino acid to be extracted. This data is also employed to re-evaluate the PA of 18C6 as the value reported in the NIST webbook is inconsistent with our experimental observations and theoretical calculations, and clearly needs to be adjusted. Trends in the computed structures and measured and calculated 18C6 binding affinities are examined to provide useful insight into the processes that occur in the molecular recognition of amino acids by 18C6 and the implications for binding to peptides and proteins.

Oral Session

Tuesday, 18th September

Room A

15:00 – 17:00

Session 17: Non-Covalent Ion-Molecule Interactions

Chair: Seung-Koo Shin (Pohang University of Schence and Technology, Korea)

S17-1620

16:20 – 16:40

Cation and solvent effects on G-quadruplex nucleic acid structure: from the solution to the gas phase

Valerie Gabelica[1], Adrien Marchand[1], Rubén Ferreira[2], Frédéric Rosu[1], Hisae Tateishi-Karimata[3], Daisuke Miyoshi[3,4], Naoki Sugimoto[3,4], Edwin De Pauw[1]

[1]University of Liege, Liège, Belgium, [2]Institute for Research in Biomedicine (IRB), Barcelona, Spain, [3]Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Japan, [4]Faculty of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, Japan

Keywords:
ion mobility spectrometry, supramolecular complexes, nucleic acids, folding, dissociation

Novel aspects:
Special DNA structures like G-quadruplexes are actually stabilized by a decrease in water activity. However, cation coordination is essential to preserve the structure from the solution to the gas phase.

Abstract:

Introduction:

"Native" mass spectrometry requires preparing the sample in solvents and buffers that preserve the desired fold, and tuning the mass spectrometer so as to just desolvate the complexes and preserve them until they reach the mass analyzer. G-quadruplex nucleic acid structures are peculiar because they require cation coordination between tetrads of guanines in order to be stable in solution. A current limitation of ESI-MS for nucleic acid studies is its incompatibility with native solution conditions, i.e. physiologically relevant salt concentrations (> 100 mM KCl, 5 mM Mg^{2+}). Ammonium ions can properly stabilize G-quadruplexes in solution, but the resulting structure may differ from the potassium-induced structure. In an effort to render the G-quadruplex structures amenable to investigation by ESI-MS more native-like, we explored G-quadruplex structural changes in ammonium solutions when electrospray-compatible co-solvents are added, thereby decreasing the water activity. This will lead us to discussing the effects of solvent (or absence thereof, i.e. gas phase conditions) and cations on G-quadruplex structure and stability.

Methods:
Oligodeoxynucleotides d (TTAGGG)$_2$, d (TAGGGT)$_2$, d (AGGGTT)$_2$ and d (GGGTTA)$_2$ were purchased from Eurogentec (Belgium). G-quadruplex dimers were formed by preparing the oligonucleotides in aqueous ammonium acetate, supplemented with various organic co-solvents (methanol,ethanol, isopropanol or acetonitrile). The water activity in these solvent mixtures was determined by the osmotic stressing method via freezing point depression osmometry using aTyp Dig. L osmometer (KNAUER, Berlin, Germany). The amount of G-quadruplex dimer formed was quantified using ESI-MS, taking into account the relative response factors [Anal. Chem., 2009, 81, 6708]. The structure of the dimer was determined in solution using circular dichroism spectroscopy on a Jobin Yvon CD 6 dichrograph, and in the gas phase using ion mobility spectrometry on both SynaptG 1 and Synapt G 2 HDMS (Waters, Manchester, UK), which were mobility-calibrated using other known G-quadruplexes. By varying the injection energy, we also probed collision-induced loss of ammonia and concomitant structural changes.

Results:
The addition of organic co-solvents to aqueous ammonium acetate promotes and accelerates the formation of dimeric G-quadruplexes from 12-mer telomeric sequences. For example, the telomeric sequence d (TAGGGT)$_2$ does not form significant amounts of dimer when annealed at 5 μM strand concentration in purely aqueous 100 mM NH_4OAc, but dimer formation is observed when common electrospray co-solvents like methanol (MeOH), ethanol (EtOH), isopropanol (iPrOH) or acetonitrile (ACN) are added to this solution. Both the total amount of dimer and the rate of dimer formation increase with the percentage of co-solvent. Interestingly, not only the fraction of co-solvent, but also the nature of the co-solvent drives the folding: conversion into parallel folds is much less favored in methanol than in the other co-solvents. We also performed a detailed thermodynamic and kinetic study of dimeric G-quadruplex formation as a function of the water activity in the different co-solvents. Faster association rates are observed in low water activity conditions, and we propose that the rate limiting factor for G-quadruplex formation is cation desolvation upon coordination to the G-quadruplex cavity. In solution as well as in the gas-phase, the dissociation rates depend on the structure in a similar way: parallel G-quadruplexes dissociate more slowly than antiparallel G-quadruplexes. We also demonstrate that ammonium ion coordination is indispensable to maintain the native structure in the gas phase.

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Room A

15:00 – 17:00

Session 17: Non-Covalent Ion-Molecule Interactions

Chair: Seung-Koo Shin (Pohang University of Schence and Technology, Korea)

S17-1640
16:40 – 17:00

Noncovalent Binding of Tetracationic Porphyrin to Thrombin-Binding Aptamer DNA Destabilizes the Guanine-Quadruplex Structure

Jongcheol Seo, Eun Sun Hong, Hye-Joo Yoon, Seung Koo Shin
Pohang University of Science and Technology (POSTECH)

Keywords:
thrombin-binding aptamer, guanine-quadruplex, hydrogen/deuterium exchange

Novel aspects:
Hydrogen/deuterium exchange experiment and infrared multiphoton dissociation spectra show that tetracationic porphyrin binds to the TGT loop of thrombin-binding aptamer DNA and destabilizes the guanine-quadruplex structure

Abstract:
A thrombin-binding aptamer (TBA) DNA d(GGTTGGTGTGGTTGG) forms the antiparallel chair-type guanine-quadruplex (G4) structure in the presence of potassium ion, which involves two stacked G-tetrads with an intercalated potassium ion. This G4 structure is an active form of the aptamer, and noncovalent interactions between negatively charged TBA and organic cation can induce a significant structural change in the G4 structure. Here we studied the accessibility of solvent to the bases of TBA in the presence or absence of tetracationic 5,10,15,20-tetrakis (*N*-methyl-4-pyridyl) porphyrin and potassium ions by hydrogen/deuterium exchange (HDX) experiment. The extent of HDX was measured by electrospray ionization (ESI) mass spectrometry (MS). The mass spectra of TBA and 1:1 TBA-porphyrin complex were obtained at various H/D ratios of ESI solvent (3:1 water/isopropanol, vol/vol) in the presence or absence of potassium ion. HDX results showed that porphyrin-bound TBA was more flexible than potassium-bound TBA or free TBA, indicating that porphyrin destabilized the G4 structure to allow easier access of solvent to the bases of TBA. To support this finding, we measured the binding constant of potassium ion to TBA and 1:1 TBA-porphyrin complex by titration method. We also examined the circular dichroism (CD) spectra of TBA. Porphyrin binding on TBA slightly reduced the potassium-binding constant from 8600 to 7900 M^{-1} and attenuated the characteristic G4 peaks in the CD spectra. These results are in line with the HDX data. Furthermore, we determined the binding site of porphyrin on TBA by infrared multiphoton dissociation (IRMPD) of the 1:1 TBA-porphyrin complex using Fourier-transform ion cyclotron resonance (FT-ICR) MS. The product ion distribution showed that the porphyrin is on the central TGT region of TBA. Thus, the planar porphyrin cation binds to the TGT loop region of the negatively charged TBA through electrostatic interactions, thereby weakening noncovalent interactions between the G-tetrads and the potassium ion and destabilizing the G4 structure of TBA.

Oral Session

Tuesday, 18th September

Room B-1

15:00 – 17:00

Session 18: Advances in Resolution and Accuracy of Isotope Ratio Analyses

Chair: Takafumi Hirata (Kyoto University, Japan)

S18-1500
15:00 – 15:40

[Keynote Lecture] Advances in Isotope Ratio Mass Spectrometry and Required Isotope Reference Materials

Jochen Vogl

BAM Federal Institute for Materials Research and Testing, Berlin, Germany

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Keywords:
reference material, traceability, measurement uncertainty

Novel aspects:
The approach of using two delta-reference materials, one at delta= 0 and one at delta=x, enables the validation of the analytical procedure and the estimation of a measurement uncertainty.

Abstract:
Outside the isotope community the isotopic composition of an element is often considered as invariant in nature, because the variations usually are too small to detect with conventional techniques. With precise mass spectrometric techniques these variations can be detected and the determined isotopic composition can be used to obtain specific information of a sample like the formation conditions, age or provenance.
In the past isotope ratio determinations to a large extent have been the domain of gas mass spectrometers and thermal ionization mass spectrometers. The invention of an inductively coupled plasma mass spectrometer (ICPMS) in the mid 1980s and its commercial introduction a few years later triggered the idea that the determination of isotope ratios will change in the future. However, it took more than a decade until competitive ICPMS instruments have been available. Especially the introduction of multi-collector (MC) ICPMS instruments and their broader availability enabled a large number of new isotope systems to be investigated.
Like any other mass spectrometric technique ICPMS is affected by mass fractionation or mass discrimination effects, which lead to a bias in the isotope ratio determination. This bias depends strongly on the type of instrument, the optimization of it and the applied measurement procedure. Therefore mass fractionation or discrimination effects need to be corrected for by applying suitable reference materials (RM) . This correction together with a proper uncertainty statement enables isotope data being traceable to the international system of units (SI) or to a commonly accepted standard. Traceability in turn enables comparability of the isotope data.
Usually this correction is carried out by applying RMs being certified for the isotopic composition of the element under investigation. Such isotope reference materials (IRMs) featuring "absolute " or "true " isotope data can only be certified via synthetic isotope mixtures, which enable the calibration of the applied mass spectrometer. This certification approach, however, is costly and labor-intensive and the achievable uncertainties are mainly limited by the purity of the enriched isotopes used. Nowadays relative standard uncertainties between 0.005 % and 0.01 % can be achieved for the isotope amount ratio of an IRM by using synthetic isotope mixtures. The reproducibility of isotope ratios determined with a MC-ICPMS of the present generation, however, can reach values of 0.001 %. It is obvious that IRMs being certified via synthetic isotope mixtures cannot fully meet the needs of present and future isotope research and therefore an alternative approach is needed.
In many fields where the interpretation of isotopic variations is of interest, isotope ratios are not required on a "true " basis, but the difference of the isotope ratio between two or more samples is of interest, which can be expressed as a δ-value. Consequently, δ-reference materials are required, preferably one material defining δ=0, the so-called δ-zero material, and one material with an offset, δ=x. It is desirable that the δ-zero material in parallel is certified for its "true " isotopic composition. The offset material is required to validate the whole analytical procedure, especially to prove the absence of isotope fractionation during the matrix separation step. As an example the situation of boron isotope measurements and available reference materials will be described. Combining both approaches, the δ-reference materials and IRM certified via synthetic isotope mixtures, the needs in isotope analysis can be met to a large extent.

Oral Session

Tuesday, 18th September

Room B-1

15:00 – 17:00

Session 18: Advances in Resolution and Accuracy of Isotope Ratio Analyses

Chair: Takafumi Hirata (Kyoto University, Japan)

S18-1540

15:40 – 16:00

An experimental study on stable isotopic fractionation of rare earth elements during the adsorption on iron and manganeseoxides

Ryoichi Nakada[1], Masaharu Tanimizu[1,2], Yoshio Takahashi[1,2]

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Keywords:
isotopic fractionation, MC-ICP-MS, XAFS

Novel aspects:
The slight change in coordination environment affects the direction and degrees of mass-dependent isotope fractionations among REE.

Abstract:
Recent development of analytical instruments, especially multiple collector (MC) ICP-MS, has enabled us to discuss the mass-dependent isotopic fractionation of heavy elements. Although several rules controlling the isotopic fractionation has have been suggested, it is still insufficient to discuss which chemical properties are the most important factor to evaluate bond stiffness in the equilibrium isotopic fractionation. This study, therefore, exhibits the results of stable isotopic fractionation of rare earth element (REE) during the adsorption experiment to discuss the cause of the isotopic fractionation among REE.
Lanthanum, Ce, Nd, and Sm chloride solutions with various concentrations were respectively added to both synthesized ferrihydrite and d-MnO_2 suspensions. In all the systems, pH was adjusted to 5.00 ($\pm$0.05) and shaken for 6 hours before the filtration. Stable isotope ratios in both liquid and solid phases were determined using MC-ICP-MS. REE-Cl_3 solutions used in the adsorption experiment were employed as standard solutions and the isotope ratio of each element was expressed in epsilon notation relative to the average standards, which is shown in the equation as follows : $\varepsilon = (R_{sample}/R_{STD} - 1) * 10^4$, where R was defined as $^{139}La/^{138}La$, $^{142}Ce/^{140}Ce$, $^{145}Nd/^{143}Nd$, or $^{149}Sm/^{147}Sm$, respectively. For the solid phase, K-edge EXAFS of filtered samples was measured at BL01B 1 in SPring-8 to obtain the information of the coordination environment.
Though accurate determination of La isotope ratio was difficult due to the large difference in the isotopic abundance between ^{138}La and ^{139}La, a broad trend obtained in this study suggested that the lighter La isotope was selectively partitioned into the solid phase. In Ce system, it is clearly shown that the lighter isotope was partitioned into solid phase, whereas in Nd and Sm systems, lighter isotopes remained in the liquid phase, suggesting that physicochemical factors have been changed between Ce and Nd. According to the EXAFS analysis, split first shell (La-O bond) was observed for La-adsorption system, suggesting that the first coordination sphere is distorted in the system. Such distortion was also expected for Ce-adsorption system from their EXAFS results. On the other hand, split first shell was not observed for Nd and Sm systems. Thus, it is expected that the slight change in coordination environment, which can also cause the difference in their hydration numbers, affects the direction and degrees of mass-dependent isotope fractionations among REE.

S18-1600
16:00 – 16:20

The influence of the extreme climate conditions on Romanian fruit juices isotopic composition

Dana Alina I Magdas[1], Romulus Puscas[1], Gabriela Cristea[1], Nicoleta Vedeanu[2]

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Keywords:
IRMS, food quality, fruit juices

Novel aspects:
The characterization of 70 Romanian fruit juices IRMS are discussed. We measured isotopic ratios to see how meteorological peculiarities of year 2011 influenced the isotopic composition of investigated fruit juices.

Abstract:
Isotope ratio mass spectrometry (IRMS) is a very useful tool for origin assignation of food, thus $^{2}H/^{1}H$, $^{18}O/^{16}O$ and $^{13}C/^{12}C$; measurements are intensively used in forensic study to prove product authenticity. This application has been particularly useful in food quality control, because it allows the detection of added sugar and water in fruit juices and in tracing the geographical origin of food. One of the greatest limitations of this technique applicability in origin assignation is the lack of large databases of isotopic abundance in food items. The interpretation of such analysis requires a sufficient number of data for authentic juices of the same seasonal and regional origin, especially since the isotopic parameters of fruit juices show remarkable variability depending on climatologically factors.
The characterization of 70 Romanian single strength fruit juices (apples, pears, plums and grapes) collected from different Transylvanian areas, Romania, by mean of stable isotope approach are presented and discussed in this work. We measured $^{2}H/^{1}H$, $^{18}O/^{16}O$ ratios from water juice and $^{13}C/^{12}C$ from pulp and we compared the isotopic compositions of fruit juices from 2010 harvest with those from 2011 one, in order to see how meteorological peculiarities of year 2011 influenced the isotopic composition of the investigated fruit juices. We correlated the $^{2}H/^{1}H$, $^{18}O/^{16}O$ isotopic ratios from water juice with those from precipitations in Transylvania aria to understand better the naturally isotopic differences that appear between these fruits produced in the mentioned two years. These differences arise from the specific meteorological conditions of year 2010, which was characterized by higher humidity in comparison with 2011. The average annual rainfall of 2010 was 57.6 l/m^2 while those corresponding to 2011 were about 37.6 l/m^2.
The obtained results have shown that beside the meteorological and climate conditions, the isotopic enrichment degree depends on the fruit species, even for the species originated from the same geographical area. The most notable differences in isotopic content appear in the case of grapes.
Our data presents significant differences in isotopic composition between fruits produced in the same areas but in two consecutive years, which are characterized by very different meteorological conditions. Thus, these results may serve in the detection of added water or C_4 type sugars (cane or corn derived sugar syrups) to commercial fruit juices.

References

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Oral Session

Tuesday, 18th September

Room B-1

15:00 – 17:00

Session 18: Advances in Resolution and Accuracy of Isotope Ratio Analyses

Chair: Takafumi Hirata (Kyoto University, Japan)

S18-1620 The Fe isotopic analysis using LAL-MC-ICPMS technique

16:20 – 16:40

Satoki Okabayashi[1], Tetsuya Yokoyama[2], Takafumi Hirata[1]

[1]Kyoto University, Kyoto, Japan, [2]Tokyo Institute of Technology

Keywords:
MC-ICPMS, laser ablation in liquid, LAL, Fe isotope

Novel aspects:
We have developed a new sampling technique, laser ablation in liquid (LAL). This technique achieve the reliable isotopic analysis using MC-ICPMS.

Abstract:
After the development of the multi-collector ICP-MS (MC-ICPMS), the accuracy and the precision of the isotopic analysis have been dramatically improved. Especially the sample-standard bracketing technique makes it much easier to produce isotopic data. To obtain the isotopic data from the solid sample, especially from the specific region or phase in the solid sample such as a mineral in a geological rock, in most cases the solid sample is crashed in the agate mortar and hand-picked or micro-mill technique is applied to dissolve the sample into the solution. It should be noted that these techniques have the risk of the contamination from the equipments or apparatus used for the sample handling. The laser ablation (LA) is one of the most sensitive sampling techniques for in-situ elemental and isotopic analysis of solid samples. The LA technique has been widely applied for the sampling technique for the ICP-MS technique to determine the isotopic compositions. The LA-ICPMS technique achieves rapid and precise isotopic analyses under the spatial resolution of better than 30 μm. Nevertheless, it should be noted that the resultant precision in the isotopic ratio measurements is significantly poorer than those achieved by the conventional solution introduction technique. Moreover, preparation of matrix matched standard is highly desired to achieve the accurate isotopic ratio measurements for complex minerals or phases. This indicates that the isotopic data obtained by the LA-ICPMS could be erroneous when no proper isotopic standards were available.

To overcome these problems, our group developed a new sampling technique, laser ablation in liquid (LAL) (Okabayashi et al., 2011 ; Douglas et al., 2011). This sampling technique is very simple and effective for the isotopic analysis using MC-ICPMS because (1) it has row risk of the contamination since any equipment does not touch the sample surface (2) the positional information can be kept during the LAL sampling procedure (3) the solution analysis is applied for the LAL-sampled material.

In this study, we have evaluated the Fe isotopic fractionation during the spot sampling. The pure Fe metal (IRMM-014) was ablated under the ~3 mm thickness deionized water. A femtosecond laser (780 nm wave length) was used for the LAL sampling. During the LAL procedure, the fluence was set to 20 Jcm^{-2} and the various repetition rates (10-250 Hz) were applied. As the result, we could not found the Fe isotopic fractionation during the spot sampling of LAL procedure. Our recent study (Okabayashi et al., 2011) also revealed that Fe isotopic fractionation did not occur during the raster sampling of LAL procedure. These results demonstrate clearly that LAL sampling technique can be applied for the isotopic analysis using MC-ICPMS.

In addition, Fe-Ni metal and FeS phase in the ordinary chondrite were sampled using present LAL technique to measure the Fe isotopic ratios of them. In the case of the LA-ICPMS technique, the reliable Fe isotopic measurement of FeS phase was difficult because of the non-spectroscopic interference from S to Fe isotopes. In this study, after the LAL sampling of the FeS phase, the suspension of the sample was decomposed by conc. HCl to volatilize S as H_2S, so the reliable Fe isotopic measurement could be achieved. The details of the Fe isotopic ratios obtained by LAL-MC-ICPMS technique will be discussed in this presentation.

Oral Session

Tuesday, 18th September

Room B-1

15:00 – 17:00

Session 18: Advances in Resolution and Accuracy of Isotope Ratio Analyses

Chair: Takafumi Hirata (Kyoto University, Japan)

S18-1640

16:40 – 17:00

A novel technique for correction of instrumental mass bias in isotope ratio measurements

Gina Chew, Thomas Walczyk

NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore

Keywords:
instrument mass bias correction

Novel aspects:
This work will open up new doors to measure fractionation effects at high accuracy that occur in nature for elements with three of more isotopes.

Abstract:
Isotope ratio measurements by inorganic mass spectrometric techniques are always subject to mass bias effects which needs to be corrected for in order to determine the true isotope ratios of the sample under study. This is necessary in particular for the measurement of small natural isotope abundance variations of metals induced by mass dependent processes in nature as an emerging tool in the Earth, Environmental and Life Sciences.

The double spike technique is the reference technique for the correction of such instrument mass bias effects in Thermal Ionization Mass Spectrometry (TIMS). It is applicable for elements with four or more isotopes. This is because there are three unknowns, namely the sample fractionation factor α, instrument fractionation factor β and the proportion of the double spike to the sample p in the blend. To solve the system, there have to be at least three isotope ratios that have the same reference isotope in the denominator. To date, there is no method available to correct for instrument mass bias effects by double spiking for elements such as magnesium which has only three stable isotopes (^{24}Mg, ^{25}Mg,^{26}Mg).

Our novel technique is based on mixing the spike with the sample in different weight ratios. The mole ratio of spike to sample will be proportional to their weight ratio and a linear plot can be established. The mole ratio for each blend is calculated following principles of isotope dilution mass spectrometry whereas the weight ratio is measured using a high-precision analytical balance. Since two isotope ratios can be measured for Mg (^{26}Mg/^{24}Mg and ^{25}Mg/^{24}Mg), the mole ratio calculated using each isotope ratio will be slightly different as the measured ratios are subject to instrument fractionation effects. The unknown variables are the instrument fractionation factor of the individual blends and the sample fractionation factor. The system is solved by finding solutions to the variables that will give the same mole ratio of spike to sample calculated from both isotope ratios for the different mixes. Optimization of the spike composition and the weight ratios for linear regression analysis was done by Monte Carlo Simulation, a numerical technique used to simulate measurement results on the basis of probability density functions (PDFs) of the input quantities. This work will open up new doors to measure fractionation effects at high accuracy that occur in nature for elements with three of more isotopes.

Oral Session

Tuesday, 18th September

Room D

15:00 – 17:00

Session 19: Mass Spectrometric Diagnosis

Chair: Toyofumi Nakanishi (Osaka Medical College, Japan)

S19-1500 [Keynote Lecture] Ambient Mass Spectrometry for Detecting Biomarkers in Breath

15:00 – 15:40

Renato Zenobi
ETH Zurich

Keywords:
Ambient MS ; SESI ; EESI ; Breath

Novel aspects:
MS-based Diagnosis by Sensitive Detection of Biomarkers in Exhaled Breath

Abstract:
Ambient mass spectrometry stands for a series of recently introduced methods for analyzing complex samples with very little or no sample preparation. Ambient MS includes methods such as Desorption Electrospray Ionization (DESI) and Direct Analysis in Real Time (DART) . We have been particularly active in the use of Secondary Electrospray Ionization (SESI) and Extractive Electrospray Ionization (EESI) for the analysis of complex liquids, surfaces (following a neutral desorption step) as well as gases and aerosols.

We have recently concentrated on breath analysis by EESI and SESI, and found the detection of exhaled drugs and metabolites with molecular weights >200 g/mol to be challenging, due to the low breath concentration. Using an ion funnel operating at ambient pressure that contains a SESI / EESI source, we are able to boost the sensitivity by a factor or 100 ... 1000, which should, for example, allow the detection of opioids in exhaled breath of anesthesized patients. Very recently, we have also employed SESI and EESI for monitoring drug dosage (in the case of valproic acid, VPA, an antiepileptic drug) and for medical diagnosis (for distinguishing patients with chronic obstructive pulmonary disease, COPD, from smokers and from healthy controls) . The latest results from these studies will be discussed.

Oral Session

Tuesday, 18th September

Room D

15:00 – 17:00

Session 19: Mass Spectrometric Diagnosis

Chair: Toyofumi Nakanishi (Osaka Medical College, Japan)

S19-1540 A Validation Assay for Cardiac Ischemia Biomarkers

15:40 – 16:00

Robert J Cotter[1], Christine Jelinek[1], Jennifer Van Eyk[1], Kevin W Meyer[2], David Taggart[3]

[1]Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA, [2]Perfinity Biosciences, West Lafayette, IN, USA, [3]John Radcliffe Hospital, Oxford, England

Keywords:
selected reaction monitoring, cardiac ischemia, biomarker, post-translational modification

Novel aspects:
Use of the post-translational modifications of albumin as biomarkers, thereby developing a test from blood that does not require albumin removal.

Abstract:
Using a triple quadrapole mass spectrometer combined with an automated sample preparation system, we have developed a selected reaction monitoring (SRM) assay to quantify Albumin PTMs (phosphorylations and cysteinylations) implicated in cardiac ischemia. The multiplex SRM assay was designed for twenty-eight HSA peptides using Skyline. Four of the most abundant transitions for each HSA peptide were selected, including one specific to the observed PTM (or lack thereof) . Relative quantification was facilitated by an internal HSA peptide and an external E.coli β-galactosidase peptide. Analyses were initially carried out on control sera to determine reproducibility and gender differences. It was then expanded to include a plasma based longitudinal mild-ischemic patient cohort. A Perfinity workstation was used to automate all sample preparation for the assay. Tryptic digestion of 1 : 50 diluted sera or plasma occured in six minutes, desalt is performed on-column, followed by a 20 minute LC-SRM analysis.

Initial results using manual sample preparation suggested that the MRM-MS approach was sufficiently sensitive to determine if any of the peptides could signal a disease pathology, though there were discrete differences between samples as a result of inconsistencies within the sample preparation pipeline. To minimize run-to-run variability, all bench-top preparation steps were eliminated and replaced with column-mediated tryptic digestion, desalting, and reverse phase HPLC separation performed in-line on a Perfinity Workstation. Incorporating the Perfinity Workstation into the assay pipeline dramatically decreased the %CVs (to under 10% for all peptides screened) . The total throughput of the assay was also increased. Sample digestion on a bench-top heat block had required 18 hours of incubation with the enzyme reaction mixture. Using an immobilized enzyme reaction chamber, complete digestion of each plasma sample now occurs in six minutes and the entire SRM-assay could be completed in less than 35 minutes, compatible with the time frame required for diagnosis. If an MI induced by cardiac ischemia remains untreated for four hours, the patient's risk of mortality increases to 90%. To validate the 28 selected HSA peptides for use as clinical cardiac ischemia markers, a fifteen patient cohort was screened. The sample cohort consists of six peripheral plasma samples per patient taken during and after aortic bypass surgery. Initial results suggest several HSA PTMs are sensitive to cardiac ischemia and may have clinical utility as ischemic biomarkers.

Oral Session

Tuesday, 18th September

Room D

15:00 – 17:00

Session 19: Mass Spectrometric Diagnosis

Chair: Toyofumi Nakanishi (Osaka Medical College, Japan)

S19-1600
16:00 – 16:20

Diagnosis, analysis of pathogenesis and therapies for transthyretin form of amyloidosis

Yukio Ando

Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto, Japan

Keywords:
amyloidosis, TTR, FAP, mass, hereditary

Novel aspects:
We have established screening system for transthyretin related amyloidosis.

Abstract:
Progress in molecular genetics and biochemical methodologies has led to the identification of various types of amyloidosis and their amyloidogenic precursor proteins. So far, 27 different amyloidogenic proteins including lactoferrin which we first reported have been identified, although the mechanism of amyloid formation in amyloidosis remains to be elucidated except for a few types of amyloidosis. One of the most important steps in the diagnosis of the specific type of amyloidosis is detection of amyloid deposits in tissues. It is not easy to predict the presence of these amyloid deposits on the basis of patients' clinical manifestations, that is, without histopathological materials, so amyloidosis is sometimes diagnosed after the death of the patients. A real-time monitoring of amyloid fibril deposition in the amyloid targeted tissues is very important.
In Japan, we have big foci of familial amyloidotic polyneuropathy (FAP) patients. As of today, reports of 123 different points of mutations or a deletion in the TTR gene have been identified. Because transthyretin (TTR) , amyloid precursor protein for FAP, is synthesized by the liver, liver transplantation has been performed for the patients to save their lives. In addition, senile systemic amyloidosis induced by wild type TTR has been focused in the recent attention. Early diagnosis of FAP is absolutely imperative for the treatments, because duration of the disease is one of the most important prognostic factors for patients' survival after treatments including liver transplantation. To make a diagnosis of FAP sooner, we established a novel rapid diagnostic system. It includes histopathological, genetic and proteomic techniques used to detect amyloid deposits in tissues, genetic mutations of the TTR gene, and variant TTR in serum. Mass spectrometric analyses can clearly detect TTR variants in the most of FAP cases and can be used for screening and double checking TTR variants with the genetic testing. Using protein chip system, presence or absence of variant TTR in serum can be screened within 3 hours. We introduce recent research progress in ATTR amyloidosis and our diagnostic system.

Oral Session

Tuesday, 18th September

Room D

15:00 – 17:00

Session 19: Mass Spectrometric Diagnosis

Chair: Toyofumi Nakanishi (Osaka Medical College, Japan)

S19-1620

16:20 – 16:40

Cerebrospinal fluid proteomics: A new window for understanding human neurological disorders

Masaya Ikegawa

Kyoto Prefectural University of Medicine

Keywords:
MALDI-TOF MS, Cerebrospinal fluid, Proteomic pattern analysis, multiple sclerosis, Parkinson's diseases

Novel aspects:
CSF proteomic pattern analysis is an effective new technology for identifying biomarker signatures of human neurological disorders such as MS and PD and their related disorders.

Abstract:
The rapid evolution of research technologies has provided novel, high-throughput tools for the investigation of intractable neurological disorders. The ability to perform in-depth analyses of genomes, transcriptomes, proteomes, and metabolomes has fueled efforts to uncover these neurological disease related targets and biomarkers. Initial evaluations of serum and cerebrospinal fluid (CSF) from case and control subjects have led to the identification of a diverse group of candidate disease associated proteins. However, being study design, sample treatment, and data complexity contributed to the significant inter study variability, the urgent demand for diagnostic, therapeutic, and prognostic biomarkers in the future care of individuals with neurological disorders has spurred novel efforts to focus these technologies on defined clinical questions. Recently, two papers[1,2] appeared from our group employing a novel proteomic strategy uncover potential diagnostic biomarkers among neurodegenerative disorders as well as demyelinating disorders. First we have succeeded in discriminating multiple sclerosis (MS) and neuromyelitis optica (NMO) by a CSF proteomic pattern analysis by using magnetic bead-based enrichment of peptides and proteins followed by matrix-assisted laser desorption / ionisation time-of-flight (MALDI) mass spectrometry[1]. In this study, the proteomic patterns of acute NMO patients, both aquaporin-4 (AQP4) seropositive and seronegative, were easily distinguished from relapsingremitting MS (RRMS) and primary progressive MS (PPMS). These distinctions were less clear, however, when CSF was obtained during disease quiescence. This finding suggests that the CSF proteome may be a sensitive barometer of CNS disease activity and therapeutic response. A smaller but measurable variability was also observed between CSF proteomes in RRMS and PPMS patients. The additional observation that the PPMS proteome may be more closely aligned with amyotrophic lateral sclerosis (ALS) than RRMS was quite intriguing and may be consistent with enhanced neurodegeneration in PPMS patients. In another study, we have succeeded in discrimination between Parkinson's diseases (PD) and multiple systemic atrophy (MSA) with the same strategy[2]. Our findings suggest that CSF proteomic pattern analysis can increase the accuracy of disease diagnosis of MS- and PD-related disorders and will aid physicians in appropriate therapeutic decision-making even for expanding neurological disease entities.

1. Komori et al., *Annals of Neurology* 2012；71：614623.
2. Ishigami et al., *Movement Disorders* 2012； 9 Mar accepted.

Oral Session

Tuesday, 18th September

Room D

15:00 – 17:00

Session 19: Mass Spectrometric Diagnosis

Chair: Toyofumi Nakanishi (Osaka Medical College, Japan)

S19-1640

16:40 – 17:00

Digging deeper into Small Cell Lung Cancer. Can targeted LC-MS reveal tomorrows diagnostic level?

Silje B Torsetnes[1], Marianne S Nordlund[2], Elisabeth Paus[2], Trine G Halvorsen[1], Leon Reubsaet[1]

[1]University of Oslo, Oslo, Norway, [2]Central Laboratory - Radiumhospitalet, Oslo, Norway

Keywords:
SRM, Triple quadrupole, SCLC, ProGRP

Novel aspects:
The combined immunocapture with the LC-MS/MS SRM detection allows determination of isoforms of the tumor marker ProGRP revealing a potential new diagnostic level in Small Cell Lung Cancer

Abstract:
The overall survival rate for Small Cell Lung Cancer (SCLC) patients is very low, less than 5 % at 5 years, despite the sensitivity of the tumors to chemotherapy and radiotherapy. The difficulty, however, is early diagnosis and monitoring of the treatment. This hampers the improvement of the disease prognosis.
Out of several possible biomarkers for SCLC, ProGRP is considered very promising, with reported qualities of high disease specificity and sensitivity. On a routinely basis, so far only the total amount of ProGRP has been measured in correlation with disease, using conventional immunometric analysis. Challenging is the very low serum concentration reported for this tumor marker (ref. level < 60 pg/mL levels) .
ProGRP comprise three known isoforms, known to be expressed on the mRNA level, but their individual prevalence has until now not been shown in serum. From a diagnostic point of view, it might be of great interest to confirm the protein expression of these isoforms as well as their presence in serum to see if ratios between them vary, e.g. between disease states or -types.
A method for total ProGRP, developed by Winther, B., et al. (1) , was used as a starting point for an improved immuno-MS method. A monoclonal antibody, E146 (2) , immobilized on magnetic beads, was used for the extraction of ProGRP-isoforms spiked to human serum. The captured ProGRP-isoforms were subjected to tryptic digestion, generating signature peptides that were further separated by liquid chromatography and detected by selected reaction monitoring (SRM) on a triple quadrupole (QqQ) mass spectrometer (MS) . Orthogonal selectivity, conferred by the combination of immunoaffinity extraction and LC-MS/MS analysis, enables development of isoform-differentiating methods.
Highly specific and accurate measurements of tumor marker concentrations, aided by LC-MS/MS, may to a larger extent than current methods give valuable clinically relevant information in diseases such as SCLC. In this presentation it will be shown that through combined benefits of three complementary separation methods, a validated method for quantifying clinically relevant concentrations of ProGRP-isoforms has been developed. Evidence of applicability on SCLC patient samples will be presented and the potential relevance of isoform differentiation will be discussed.

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2. Nordlund, M. S., Fermer, C., Nilsson, O., Warren, D. J., and Paus, E. (2007) Production and Characterization of Monoclonal Antibodies for Immunoassay of the Lung Cancer Marker proGRP, Tumor Biol 28, 100-110.

Oral Session

Tuesday, 18th September

Room E

15:00 – 17:00

Session 20: The Ion formation and Dissociation Mechanisms in MALDI

Chair: Myung Soo Kim (Seoul National University, Korea)

S20-1500

15:00 – 15:40

[Keynote Lecture] MALDI and Related Desorption / Ablation / Ionization Methods:A Solved Problem or Still a Mystery?

Richard Knochenmuss

Tofwerk, Thun, Switzerland

Keywords:
MALDI Ionization Ablation

Novel aspects:
Keynote on MALDI ionization mechanisms

Abstract:
MALDI is one of the key technologies of modern mass spectrometry. The ablation and especially ionization mechanisms underlying it have therefore been the object of much speculation and many studies. With the recent development of variants and alternatives to MALDI and electrospray, more mechanistic questions have arisen, as well as questions of similarities between the methods.

A brief, critical overview will be presented of the development of MALDI mechanisms. A recurring theme is the hope that a simple basic mechanism largely explains MALDI, followed by disappointment as reality is found to be more complicated. This will be followed by a summary of the current status of MALDI models, both qualitative and quantitative. The author's models will be discussed in deeper detail. Some related recent methods will be compared, and parallels discussed. A common aspect is a rapid, nonequilibrium phase change, during which multiple interconnected ion-molecule reactions take place. Some opinions on what is solved and what remains mysterious may be offered.

Oral Session

Tuesday, 18th September

Room E

15:00 – 17:00

Session 20: The Ion formation and Dissociation Mechanisms in MALDI

Chair: Myung Soo Kim (Seoul National University, Korea)

S20-1540

15:40 – 16:00

Ion yield and laser shot number-dependent variation of mass spectral pattern in MALDI of peptides; a thermal model for MALDI

Yong Jin Bae, Kyung Man Park, Sung Hee Ahn, Myung Soo Kim
Seoul National University, Seoul, Korea

Keywords:
MALDI, Ion yield, Thermal model

Novel aspects:
MALDI-TOF spectra for peptides became highly reproducible when a homogenous sample prepared by vacuum-drying was used and when the spectra displaying the same 'early' plume temperature were compared.

Abstract:
The mechanism for the formation of gas-phase peptide ions in matrix-assisted laser desorption ionization (MALDI) and the factors determining mass spectral patterns in MALDI-TOF of peptides were investigated. α-Cyano-4-hydroxycinnanmic acid (CHCA) was used as the matrix. The main tools for the study were the measurements of the ion yields (IY, number of ions formed ÷ number of molecules in a sample) and the laser shot number-dependence of the spectral pattern. A rather homogeneous sample was prepared by vacuum-drying of water/acetonitrile solution of a peptide/CHCA mixture, rather than the usual air-drying.
IYs were low 10^{-4} for peptides and 10^{-7} for CHCA. Both of these were smaller than those speculated by Hillenkamp and Karas by two orders of magnitude. Total number of ions in a spectrum, i.e. the sum of peptide- and matrix-derived ions, was the same regardless of peptides and their concentrations. Namely, the number of ions generated from a pure matrix was the upper limit to the number of peptide-derived ions. The total number of ions was unaffected by the laser pulse energy, invalidating laser-induced ionization of matrix molecules as the mechanism for the primary ion formation. Instead, the excitation of matrix by laser seems to be a way of supplying thermal energy to the sample. Accepting strong Coulomb attraction felt by cations in a solid sample, we propose three hypotheses for gas-phase peptide ion formation. In Hypothesis 1, they originate from the dielectrically screened peptide ions in the sample. In Hypothesis 2, the pre-formed peptide ions are released as part of neutral ion pairs, which generate gas-phase peptide ions via reaction with matrix-derived cations. In Hypothesis 3, neutral peptides released by ablation get protonated via reaction with matrix-derived cations.
By repeatedly irradiating a spot on a sample with laser, a set of mass spectra was collected. For each spectrum, the temperature in the early plume (T_{early}) was estimated by kinetic analysis of the peptide ion dissociation yield. T_{early} decreased as the shot continued because thermal conduction at the irradiated spot became more efficient as it got thinner. At a given T_{early}, the peptide-to-matrix ion abundance ratio was proportional to the peptide-to-matrix ratio in the solid sample. It increased as the shot continued. These indicate that the exothermic matrix-to-peptide proton transfer was in quasi-thermal equilibrium in the early matrix plume. For samples with the same peptide-to-matrix ratio, the overall spectral pattern was the same regardless of the experimental condition when the spectra with the same T_{early} were compared, suggesting thermal determination of MALDI spectral pattern.

Oral Session

Tuesday, 18th September

Room E

15:00 – 17:00

Session 20: The Ion formation and Dissociation Mechanisms in MALDI

Chair: Myung Soo Kim (Seoul National University, Korea)

S20-1600

16:00 – 16:20

Plume Expansion Dynamics of Ionic Liquid Matrices for Matrix-assisted Laser Desorption/Ionization

I-Chung Lu, Sheng Lee, Yuan T Lee, Chi-Kung Ni

Academia Sinica, Taipei, Taiwan

Keywords:
Ionic liquid, MALDI, matrix,

Novel aspects:
High-resolution angular and velocity distribution of ionic liquid and solid matrices are obtained to describe expansion dynamics of MALDI

Abstract:
Ionic liquid has been used as MALDI matrix in recent years. Comparing to conventional solid matrices, it shows homogeneous sample preparation, higher analyte signal intensity, and wider detection mass range. In this study, we used modified crossed-molecular beam apparatus to measure the angular, velocity, and mass distributions of desorbed neutral products from the irradiation of 355 nm on ionic liquid matrix. We investigate the effect of amine (n-Butylamine, IMTBA) as additive base for the commonly used solid matrix α-Cyano-4-hydroxycinnamic acid (CHCA). The results show that the desorbed neutral molecules from ionic liquid matrix are more than ten times larger than that from conventional solid matrix. The angular, velocity, and mass distribution measurements provide the clues to understand the MALDI mechanism of ionic liquid.

S20-1620 New Insights in MALDI In-Source Decay Process

16:20 – 16:40

Daiki Asakawa[1,2], Nicolas Smargiasso[1], Edwin De Pauw[1]

[1]University of Liege, Liege, Belgium, [2]Yokohama City University, Yokohama, Japan

Keywords:
In-source decay, Initial velocity, Internal energy, Hydrogen transfer

Novel aspects:
Consider the ions initial velocity and the presence of O-centered radicals in MALDI plume allows better understanding the MALDI-ISD process.

Abstract:
Introduction : Matrix-assisted laser desorption/ionization in-source decay (MALDI-ISD) has been utilized as a tool for top-down protein identification. The major MALDI-ISD fragments are the *c*'and *z*' ions which are originated from the N-C$_\alpha$ bond cleavage via hydrogen transfer from matrix to peptide. [1] In contrast, unexpected fragments, such as *a*', *y*' and *w* ions, are also observed. Those fragments cannot be explained by the N-C $_\alpha$ bond cleavage alone and their origin is studied in this study using three different matrices, 2,5-dihydroxybenzoic acid (2,5-DHB) ,2-aminobenzoic acid (2-AA) and 1,5-diaminonapthalene (1,5-DAN) . The influence of the initial velocity and the internal energy of analyte ions on fragment ion yield in MALDI-ISD processes is described.

Experimental : Analyte peptides were dissolved in water at concentration of 20 pmol/μl. Matrices were dissolved in water/acetonitrile (1 ; 1, v/v) with 0.1 % formic acid. The each 0.5 μl of matrix and analyte solutions were deposited on the MALDI plate and allowed to dry at room temperature. MALDI-ISD mass spectra were recorded using a time-of-flight mass spectrometer, UltraFlex II (Bruker Daltonics, Germany) equipped with freauency-tripled Nd : YAG laser (355 nm) .

Results : MALDI-ISD generates *c*' and *z*' ions by the radical-induced cleavage at the N-C$_\alpha$ bonds. The [*z*+matrix] fragments were also generated, whereas the matrix adducts bound to *c*' ions were not observed. Therefore, MALDI-ISD processes may form the *c*' /*z*· fragment pair, and the *z*· radical fragments then gain a hydrogen atom or react with the matrix. The *z*' and *w* ions originate from z· radical. These reactions competitively occur during MALDI-ISD processes. The hydrogen attachment reactions of *z*· radicals must occur by the collision between *z*· radicals and matrix molecules in the MALDI plume. Therefore, it is suggested that the ion yield of *z*' depends on the collision rate of analyte molecules in the MALDI plume. According to desorption model described by Spengler and Kirsch, the collision rate is proportional initial velocity of analytes. [2] It is known that the initial velocity of analytes, v_{in}, is dependent on the matrix. [3] The order of initial velocity is 2,5-DHB > 2-AA > 1,5-DAN. The use of 1,5-DAN gave high ion yields of *w* ions, while the use of 2,5-DHB gave low ion yields of *w*ions. The order of ion yield of *w* ions is 1,5-DAN > 2-AA > 2,5-DHB, which is inversely proportional initial velocity of analyte ions. When the 1,5-DAN is used, the high abundance of *w* ions compared when using 2,5-DHB can be understood as resulting from low collision rate in the MALDI plume allowing unimolecular dissociation. We found that MALDI-ISD with 1,5-DAN can be a useful method for the discrimination of Leu and Ile residues.
MALDI-ISD also generated *a*·/*y*' ions. Their intensity increases with the internal energy of analyte ions. As they cannot be explained by the N-C$_\alpha$ bond cleavage, it is suggested that the formation of *a*·/*y*' fragment pair is initiated by thermal activation of carbon-centered radical, leading to an oxygen-centered radical. Subsequent radical-induced fragmentation leads to *a*·/*y*' fragment pair. The relative importance of the *a*·/*y*' formation increases with decreasing proton affinity of matrix.

References
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Oral Session

Tuesday, 18th September

Room E

15:00 – 17:00

Session 20: The Ion formation and Dissociation Mechanisms in MALDI

Chair: Myung Soo Kim (Seoul National University, Korea)

S20-1640

16:40 – 17:00

CO2-Laser Atmospheric Pressure Ionization of Acoustically Levitated Droplets

Arne Stindt[1], Merwe Albrecht[1], Ulrich Panne[1,2], Jens Riedel[1]

[1]BAM Federal Institute for Materials Research and Testing, Berlin, Germany, [2]Humboldt-Universitaet zu Berlin, Department of Chemistry, Berlin, Germany

Keywords:
Ultrasonic Levitation, AP-Ionization, Lucky Survivor

Novel aspects:
A simplified ionization scheme for levitated droplets was established. Besides its direct practical applicability it helps understanding classical ionization mechanisms.

Abstract:
Once designed for zero gravity experiments, in recent years levitation devices have experienced a renaissance in microfluidics where they serve as wallless reactors and for contactless sample handling. Until now many optical analytical techniques have been employed to interrogate the contents of levitated droplets, however only one approach towards a coupling to mass spectrometry has been successfully conducted utilizing a combination of corona charging, pulsed high voltage electrodes, MALDI matrix addition to the droplet and desorption with an UV-laser [1].
In the presented work we used a single CO_2 laser pulse for vaporization and ionization out of previously uncharged droplets following the approach of CO_2 MALDI proposed by Hillenkamp 20 years ago [2]. We used water as solvent, glycerol as IR chromophore and amino acids and small peptides as analytes. The resulting spectra resemble results expected for MALDI conditions and show no fractionation products. This soft ionization is attributed to the low energy coupled to the system by individual photons dictating mere dynamics in the electrical ground state. The observed ions therefore corroborate the lucky survivor mechanism postulated by Karas *et al.* [3]. In order to visualize the vaporization and plume formation, the experiments were accompanied by high repetition rate shadowgraphy.

References

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[2] A. Overberg, M. Karas, F. Hillenkamp, *Rapid Commun. Mass Spectrom.*, **1991**, *5*, 128-131.
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Poster Session

Tuesday, 18th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 11: Glycomics: From Disease Markers to Therapeutic Antibody Products

PTu-001

11:10 – 12:20

Identification of Native N-linked Glycan Structures from Human Cancer Cell Using HPLC on a Microfluidic Chip and Time-of-Flight Mass Spectrometry

Jong-Moon Park[1], Kyeong-seob Lee[4], Na-Young Han[1], Eugene C Yi[3,4], Hookeun Lee[1,2]

[1]Lee Gil Ya Cancer and Diabetes Institute, Incheon, Republic of Korea, [2]College of Pharmacy, Gachon University, Republic of Korea, [3]Dept of Molecular Medicine and Biopharmaceutical Sciences Seoul National University, College of Medicine, Republic of Korea, [4]Dept.of Molecular Medicine and Biopharmaceutical Sciences Graduate school of Convergence Science and Technology Seoul National University, Republic of Korea

Keywords:
Glycan, Q-TOF

Novel aspects:
N-linked glycan analysis

Abstract:
Glycan play key roles in protein folding, cell-cell recognition, cancer metastasis, and the immune system. In cell, glycosylation comprise that many kinds of glycan structures associated with a specific glycoprotein have different functions. So, the biological meaning of glycosylation has made them a prime target for discovering biomarker in diseases. Identifying the numerous glycan, we have used new method to assess the diversity of the N-linked oligosaccharides without derivatization has been developed using on-line nano-liquid chromatography (nanoLC) and high resolution time-of-flight mass spectrometry. In three times MS/MS results, chips offered good sensitivity and reproducibility in separating a intact protein. In tandem mass spectra, glycan fragment ions were identified asN-glycan sequences for mining glycoproteome. As a result, we have identified twelve N-glycan structures from murine myeloma recombinant Human epidermal growth factor receptor (EGFR) .

PTu-002 Development of Analytical Method of Keratan Sulfate Structure

13:30 – 14:40

Shigeyasu Ito, Hiroshi Fujita, Toshikazu Minamisawa

SEIKAGAKU CORPORATION, Tokyo, Japan

Keywords:
keratan sulfate, oligosaccharide, marker, diagnosis, LC/MS

Novel aspects:
We have successed in developing an advanced profiling method for oligosaccharides of keratan sulfate from different biosources.

Abstract:
Keratan Sulfate (KS) is known as a linear polymer of *N*-acetyllactosamine, 3 Gal β 1 - 4 GlcNAc β 1. The majority of the GlcNAc and a significant proportion of the Gal are sulfated at the C 6 position, and it was also reported that fucosylation and/or sialic acid end-capping can occurred. This polymer is attached to cartilage proteoglycans such as aggrecan and fibromodulin. Several studies have been reported that structural changes, such as sulfation degree, sialylation and fucosylation was occurred on KS in cartilage during maturation[1)-3)]. Besides, it was confirmed that the serum concentration of KS correlated with the severity of the damage of articular cartilage[4)]. However, there are few reports that studied about the structural changes of cartilage and/or serum KS molecules in such diseases as osteoarthritis and intervertebral disk degeneration. In order to investigate this subject, we constructed a high sensitivity evaluation system, which enables to detect a small amount of KS, and attempted to analyze whether the difference between two KSs derived from different origins becomes clear or not, using the system.

KS isolated from bovine cornea (BKS) and from shark cartilage (KPS) were used as model samples. These samples were thoroughly digested with Keratanase II. Generated oligosaccharides were separated on a Superdex 75 10/300 GL (GE healthcare) with 50% ethanol as a running solution. Structural and quantitative analysis of these oligosaccharides were performed using LCMS-IT-TOF system (Shimadzu) equipped with nanoelectrospray ionization source with negative ion mode.

KS degradation products were detected in trace amounts which were assumed to be several ten micrograms. It was revealed that more than 70 % of the generated compounds were disaccharides in each KS sample. Other oligosaccharide structures, presumed to be sialylated or de-acetylated, could also be observed, and the amount varied between BKS and KPS. In this way, analysis of KS oligosaccharide structures and the amounts by this sensitive system was considered promising to provide information on sulfation, sialylation and de-acethylation pattern of KS which change could reflect the cartilage disease.

References

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PTu-003
11:10 – 12:20

Detection of the Heterogeneous O-Glycosylation Profile of MT1-MMP Expressed in Cancer Cells by a Simple MALDI-MS Method

Takuya Shuo[1], Naohiko Koshikawa[1], Daisuke Hoshino[1], Tomoko Minegishi[1], Hiroko Ao-Kondo[2], Masaaki Oyama[2], Sadanori Sekiya[3], Shinichi Iwamoto[3], Koichi Tanaka[3], Motoharu Seiki[1]

[1]Division of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Medical Proteomics Laboratory, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [3]Koichi Tanaka Mass Spectrom. Res. Lab., Shimadzu Corp., Kyoto, Japan

Keywords:
MALDI-MS, Liquid matrix, On-target separation, Glycoprotein, Cancer cell

Novel aspects:
A single droplet of a whole protein digest applied to a liquid matrix 3AQ/CHCA on a MALDI target plate was sufficient to identify glycosylated and nonglycosylated peptides.

Abstract:
Glycosylation is an important and universal post-translational modification for many proteins, and regulates protein functions. In particular, specific changes in protein *O*-glycosylation have been implicated in malignant tumors and therefore development of a simple method to determine *O*-glycosylation of limited amounts of individual proteins has been desired. MALDI-MS is an indispensable analytical tool for elucidating both the peptide and glycan moieties of glycopeptides. However, it has been difficult to analyze glycosylated and nonglycosylated peptides within a proteolytic digest simultaneously by MS because the ionization efficiency of glycosylated and nonglycosylated peptides is so different. Although several methods have been developed for isolation of *O*-glycopeptides, they are time-consuming and give rise to poor yields. A new on-target separation technique to analyze glycopeptides in a highly sensitive manner by MALDI-MS using the liquid matrix 3AQ/CHCA, which is composed of 3-aminoquinoline and alpha-cyano-4-hydroxycinnamic acid, was developed recently [Sekiya et al. (2012) *Rapid Commun. Mass Spectrom.* 26, 693-700] . In this method, a single droplet of proteolytic digest of a protein sample containing both glycosylated and nonglycosylated peptides is spotted directly onto the surface of the liquid matrix 3AQ/CHCA on a MALDI target plate. During evaporation of solvents of the digest on the matrix, hydrophilic constituents such as glycopeptides are concentrated within the central area. In contrast, hydrophobic peptides tend to be excluded from the evaporating solution and are left at the periphery of the liquid matrix.

Here we optimized the technique to analyze a small amount of transmembrane protein separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) , which is a common procedure for the small-scale separation of proteins. Then we applied the MALDI-MS method to evaluate the *O*-glycosylation profile of membrane-type matrix metalloproteinase MT1-MMP expressed in cancer cells at a low level and tried to detect the heterogeneous modification status. MT1-MMP is a potent invasion promoting protease expressed on the surface of aggressive cancer cells. *O*-glycosylation of MT1-MMP is reported to modulate its protease activity and thereby to affect cancer cell invasion. MT1-MMP expressed in human fibrosarcoma HT1080 cells was immunoprecipitated and resolved by SDS-PAGE. After in-gel tryptic digestion of the protein, a single droplet of the digest was applied directly to the liquid matrix 3AQ/CHCA on a target plate and analyzed by a digital ion trap-type MALDI-MS. The tryptic MT1-MMP digest was allowed to evaporate and the central portion of the sample load area was irradiated using a laser beam. The MS spectra generated comprised several protonated ion $[M + H]^+$ peaks and the distances between the peaks corresponded precisely to the masses of typical monosaccharides : 162 and 203 Da for hexose and *N*-acetylhexosamine, respectively. The collision-induced dissociation of these peptide peaks via MS^2 analysis indicated that the fragment ions correspond to a series of losses of monosaccharides from a single peptide glycosylated differentially. The glycoforms of this peptide contained 2 to 5 hexose and *N*-acetylhexosamine. Fragment ion spectra were also obtained from another glycopeptide as a sodiated ion $[M + Na]^+$ peak. On the other hand, many unmodified peptides derived from the MT1-MMP digest were detected in the periphery of the liquid matrix. Overall, we achieved approximately 50% sequence coverage of MT1-MMP using this method. Thus, a whole protein analysis of glycosylated and nonglycosylated peptides was accomplished following conventional SDS-PAGE, in-gel digestion, and direct application of a single droplet of a sample digest onto the liquid matrix 3AQ/CHCA on the MALDI target plate.

PTu-004
13:30 – 14:40

Age-related Changes in the Composition and Structure of Glycan released from Mouse Skin Tissues

Bum Jin Kim[1], Inae Kang[1], Su Hee Kim[1], Sureyya Ozcan[2], Lauren Dimapasoc[2], Ik-Soon Jang[3], Jong-Soon Choi[3], Hyun Joo An[1]

[1]Chungnam National University, Daejeon, Republic of Korea, [2]Department of Biochemistry, University of California Davis, Davis, CA95616, [3]Korea Basic Science Institute, 113 Gwahangno, Yuseong-gu, Daejeon, Korea

Keywords:
Aging, N-glycan, O-glycan, Aging biomarker, Mass spectrometry

Novel aspects:
Rapid, sensitive, and quantitative monitoring for the change of glycans during aging by mass spectrometry

Abstract:
Glycosylation is highly sensitive to the biochemical environment and glycans on a protein play an important role in cell development. Aging changes have been found in all of the body's cells, tissues, and organs. These changes affect the functioning of all body systems. Thus, monitoring of the change of glycans during aging may be new approach for aging biomarker discovery. Mass spectrometry was employed for this study because it is particularly fit for quantitative and qualitative analysis of glycans due to its intrinsic speed and sensitivity. Here, we have monitored the alteration of glycans released from mouse skin tissues with chronological aging by high performance mass spectrometry.

Mouse skin tissues (three mice sets of 3, 6, 9, 12, 15, 18, 21 and 24 months) were cut about 20mm diameter and ground into small dices with 70 % ethanol. Ground tissues were dialyzed against water for 24 hours. Supernatant containing glycans were readily collected. N-glycan were enzymatically released using PNGase F for overnight in a water bath at 37&#8451 ; . O-glycans were chemically released by β-elimination with sodium borohydride ($NaBH_4$) . Released glycans were purified and fractionated by solid phase extraction using a porous graphitized carbon. Enriched glycans were profiled by MALDI-TOF/TOF mass spectrometry. Quantitative analysis and structure elucidation of glycans were further performed by Nano LC-chip Q-TOF mass spectrometry. PCA analysis of glycans was also performed to examine the alteration of glycosylation during natural aging.

We have profiled several N-glycans including high mannose and neutral-complex type by MALDI-TOF/TOF mass spectrometry. Fucosylated glycans consisting of only Hex, HexNAc, and Fuc were highly abundant in both young (6 months) and old (21 months) mouse skin tissues. Tandem mass spectrometry were further performed to assign the structure of glycans. O-glycans released from skin tissues of mouse aged from 3 months to 24 months were analyzed by mass spectrometry. Approximately 40 glycans (composition only) were identified in mouse skin tissues. Of them, 12 O-glycans having O-glycan cores were reselected for further analysis. Interestingly, we observed two distinct O-glycans : *m/z* 693.2586 ($Hex_1HexNAc_1NeuGc_1$) and *m/z* 751.3024 ($Hex_2HexNAc_2$) . The abundance of two glycans gradually were changed during natural aging. At first, the abundance of ion at *m/z* 693.2586 was readily increased from 3 months to 12 months and decreased since 15 months. The abundance of another ion at *m/z* 751.3024 also shows very similar change pattern like *m/z* 693.2586, which suggests two glycans follow same glycosylation biosynthesis pathway. Two selected O-glycans were further explored to obtain isomer specific structure information using Nano LC-chip Q-TOF mass spectrometry. The first ($Hex_1HexNAc_1NeuGc_1$) has three isomers (RT : 8.363 min, 8.799 min, and 8.995 min) , while the latter ($Hex_2HexNAc_2$) has two isomers (RT : 10.293 min and 10.391 min) . In addition, we could identify linkage and structure isomers by tandem mass spectrometry. Glycans at *m/z* 693.2586 ($Hex_1HexNAc_1NeuGc_1$) are linkage isomers which have same structures with a different linkage between monosaccharide, thus, they have same fragments. Structure isomers can be easily identified by tandem mass spectrometry. Glycans at *m/z* 751.3024 ($Hex_2HexNAc_2$) are structure isomers containing unique '*finger printing*' fragments. We will explore more glycans released from mouse skin tissues to examine the change during natural aging.

PTu-006
13:30 – 14:40

An MSn platform for detailed characterisation of both the peptide and the glycan moieties and the peptide/glycan linkage in glycoproteins.

Helen V Montgomery[1], Matthew E Openshaw[2], Omar Belgacem[2], Masaki Murase[3], Koichi Tanaka[3]

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Keywords:
glyoprotein, glycopeptide, MALDI, MSn, characterisation

Novel aspects:
Complete MSn characterisation of glycoproteins, including peptide sequence, glycan isomeric structure and peptide/glycan linkage using a MALDI-QIT-TOF Mass Spectrometer and novel software solutions.

Abstract:
The analysis of glycoproteins has become essential in light of the recent developments in the biosimilars /follow-on biologics market. As increasing numbers of pharmaceutical companies have begun research in the field of therapeutic proteins and peptides, competition has increased in terms of creating and manufacturing recombinant versions of biologically active proteins using *E Coli*, yeast, human or CHO cell expression models. EU and FDA regulation regarding what may be classified as a biosimilar is very strict and requires extensive characterisation of the recombinant protein, in particular the glycosylation sites, the glycan structure and the protein sequence.

The platform presented here combines MS^n acquired on a MALDI-QIT-TOF mass spectrometer and dedicated software tools : SIMSE[1] (Shimadzu Corp., Japan) for glycopeptides characterisation and localisation of the peptide glycan linkage and Accurate Glycan Analyser[3] - AGA (Shimadzu Corp, Japan) for glycan structural identification.

CID of N-linked glycopeptides exhibits a characteristic cleavage pattern (triplet of peaks) that corresponds to a single HexNAc molecule bound to the peptide (peptide + 203 Da) , the peptide linked to a ring cleaved HexNAc ($^{0,2}X$) (peptide + 83Da) and the peptide with no glycan[2]. This triplet of peaks is used by SIMSE to determine the respective molecular weights of the glycan and the peptide moieties. Furthermore software guided de-novo sequencing within SIMSE provides the peptide sequence. However, SIMSE can only provide the general composition of the glycan, not the precise structure and linkages. For structural isomer characterisation of the glycan moiety, AGA software uses MS to MS^4 analyses and a populated database of real MS and MS^n glycan spectra. Determination of the correct glycan structure is achieved using a combination of the MS and MS^n data, relative intensities of the fragment ions and a probabilistic scoring scheme to differentiate isomeric glycan structures.

Glycoproteins were reduced and alkylated prior to tryptic digestion and glycopeptide enrichment. The enriched sample was analysed by MS^2 and MS^3 and these data were investigated using SIMSE software. In parallel, the enriched sample was treated with PNGase F and the resulting glycans isolated using a combination of C18 and graphite materials and labelled with amino-pyridine. These purified glycans were analysed using AGA software-directed multi-stage MALDI mass spectrometry combining upto MS^4 spectra to unambiguously identify the specific glycan isomeric structure.

Preliminary MS, MS^2 and MS^3 data using the tryptic digest of the standard glycoprotein Human Transferrin provided the complete peptide sequence of the glycopeptides GP 2 (QQQHLFGS**N**VTDCSGNFCLFR ; m/z 4137) . SIMSE identified the characterisitic triplet peak for the localisation of the peptide/glycan bond (Asn 630) , as well as the general composition of the glycan (Hex 5, HexNAC 4) deduced from the glycan moiety molecular weight and fragmentation of the whole glycopeptide. A combination of MS and MS^2 of the PNGaseF cleaved glycan using AGA provided the precise bi-antennary glycan structure for transferrin GP2. This method was applied to the characterisation of other biologically relevant glycoproteins.

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PTu-007
11:10 – 12:20

Comparative Study of Protein Concentration, Glycoform Profile and Site Occupancy of a Glycoprotein with Multiple Glycosylation Sites and Complex Glycans

Yi-Chun Ma, Guor-Rong Her

National Taiwan University, Taipei, Taiwan

Keywords:

Glycoprotein quantitation ; glycosylation site occupancy ; glycan profile ; mass spectrometry ; isotope labeling

Novel aspects:

This is the first strategy capable of differentiating the variation of a glycoprotein which has multiple glycosylation sites bearing complex type glycans.

Abstract:

Over half of human proteins are glycoproteins. The glycoprotein concentration, glycosylation site occupancy and glycan profile may vary with regulatory states, types of diseases and even with disease progression; therefore, comparative analysis may provide useful information. A strategy was proposed for comparative analysis of glycoproteins in which the change of protein concentration, the change of glycosylation site occupancy and the change of site-specific glycoform profile could be differentiated and determined. To demonstrate the utility of the strategy, the glycoprotein asialofetuin which has multiple glycosylation sites bearing complex type glycans was analyzed. Comparative analysis was performed using stable isotope labeling of glycopeptides and peptides by formaldehyde-D_0 and formaldehyde-^{13}C, D_2 and analyzed by RPLC-ESI-MS. Glycopeptide signals were identified by the largely present glycosidic fragments in the MS/MS spectra. Dimethyl labeling reaction of glycopeptides was examined to be complete. The relative intensity of nonglycosylated peptides provided information about protein concentration variation. Change of site-specific glycoform profile was obtained by the relative glycoform profile of D_0-dimethyl and ^{13}C, D_2-dimethyl labeled glycopeptides. By knowing the variation of protein concentration and the variation of site-specific glycoform profile, the glycosylation site occupancy could be obtained.

PTu-008
13:30 – 14:40

Automated intact mass analysis for routine identity testing and quality tracking of recombinant monoclonal antibodies

Yoshihiko Morishita[1], Jette Wagtberg[2], Christian Albers[3], Laura Main[3], Wolfgang Jabs[3]

[1]Bruker Daltonics K.K., [2]Symphogen A/S, Lyngby, Denmark, [3]Bruker Daltonik GmbH, Bremen, Germany

Keywords:
Antibody, protein, QC, glycosylation

Novel aspects:
Fully automated solution for the characterization of antibodies. Automated platform for identity testing

Abstract:
Symphogen is developing recombinant antibody mixtures, a new class of antibody therapeutics for the treatment of serious human diseases. Development of such products presents a number of challenges, including the development of effective methods for manufacturing and release of a vast number of recombinant antibodies as well as mixtures. Characterization methods and identity tests represent key analytical methods that need to be established during development.

To ensure the correct sequence and identity of batches throughout development from discovery to tech transfer for GMP production, an intact mass analysis has been implemented as identity test. The same analysis is used for characterization to evaluate fragmentation and glycosylation pattern. Initially, this method was time consuming due to the high number of samples and the need for hands-on data processing. In addition, a system was needed for tracking data on older batches and for comparative analysis. However, with the implementation of newly developed software, we are able to run intact mass both as an identity assay and for characterization, in a fast and automated manner, including all steps from sample injection to reporting results in one seamless workflow.

The glycosylation pattern of antibodies is an important quality attribute, as it potentially affects antibody potency and clearance. The overall glycosylation pattern is influenced by changes in the upstream and downstream process, as well as individual production clones gives rise to glycosylation variations. Therefore, a workflow for fast and cost-effective evaluation of change in glycosylation is crucial during process development and clone selection. We demonstrate data in visually easy to understand views and reports that show how our new method enabled us to guide process development and clone selection.

Besides quality assessment of glycosylation patterns, we use the new methodology in high throughput identity assays, where a minimum of 75 samples can be analyzed in a day including automated data processing and data evaluation. We show here how with a searchable database of processed results, it is easy to access old data, and/or track quality over time for a specific antibody, process or clone during preclinical development.

PTu-009
11:10 – 12:20

Comprehensive Glycan Profiling of Exosomes Derived from Human Body Fluids for Disease Biomarker Discovery

Seunghyup Jeong[1], Do-Young Choi[2], Pyong-Gon Moon[3], Kwang Pyo Kim[2], Moon Chang Back[3], Hyun Joo An[1]

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Keywords:
Exosomes, microvesicles, glycan, glycosylation, biomarker

Novel aspects:
The first study of comprehensive glycan profiling of exosomes using mass spectrometry.

Abstract:
Exosomes (or microvesicles) are secreted by various cell types including tumor cells. They are composed of membrane, cellular proteins and RNA derived from their parent cells. They play an important role as mediators in extracellular communication. It is also known that exosomes are involved in tumor metastasis, angiogenesis, and antitumor immunity in cancer cells. However, the glycosylation of exosomes has not yet been studied. In this study, we have analyzed N-glycans derived from two types of exosomes, namely cancer cells and urine. First, we developed the method to profile exosome glycans by mass spectrometry. Using newly developed analytical method, glycans of exosomes derived from several lung cancer cell lines (PC9, PC 9 /R, A549) were profiled. Then we compared exosome glycans with glycans on their parent cell membranes to examine glycosylation correlation between origin cells and their exosomes. Furthermore, we have profiled glycans released from urinary exosomes of hematuria patients and control group (n=18) to examine the difference of glycosylation between two sample groups for biomarker discovery. All exosomes (about 70ug each) were treated with PNGase F to release glycans without any further sample preparation. Released glycans were then enriched by solid phase extraction (SPE) using a porous graphitized carbon (PGC) cartridge and solvents of varying polarity (different concentrations of aqueous acetonitrile) to partition them into highly anionic and neutral components. The fractions were then analyzed by two types of mass spectrometry. Ultraflextreme MALDI-TOF/TOF MS (Bruker daltonics) and Nano LC-chip Q-TOF MS (Agilent technologies) were used for overall glycans profiling and quantitation, respectively.
Interestingly, we found that glycans of exosomes derived from cancer cell lines contain over 75 % of high-mannose glycans consisting of trimannosyl core and $[Man]_{5-9}$. The most abundant glycan is Man 8 (*m/z* 1743.581, Hex_8 $HexNAc_2$) . While complex / hybrid type glycans with at least one fucose account for 25%.
In order to study the differences of glycosylation between wild type exosomes and anticancer drug Gefitinib (Iressa, EGFR antagonist) resistance type, we compared exosome glycans between PC 9 and PC 9 /R. Total abundance of glycans on PC 9 is slightly higher than that of PC 9 /R although the number of glycans analyzed by LC/MS is quite similar. We performed bio- and analytical replica to validate our preliminary data. In addition, we further analyzed N-glycans from more exosomes derived from various cancer cell lines and their origin cancer cells, respectively to examine the correlation if cancer cell lines and their exosomes have similar glycan profile.
In hematuria patient, IgAN (IgA nephropathy) develops end-stage renal disease and TBMN (Thin basement membrane nephropathy) excellent outcome which cannot be considered a disease. These two diseases have same symptom and are only diagnosed through a biopsy. This traditional way is invasive. Here, we have studied to investigate non-invasive diagnosis tool focused on patient's urine. Urine sample can be easily obtained. Using newly developed method glycans have been released from exosomes prepared from 18 clinical samples consisting of one control group (n= 6) and two hematuria patient groups (IgAN (n= 6) , TBMN (n= 6)) . Glycans between groups will be further examined for biomarker discovery.

PTu-010
13:30 – 14:40

Determination of core fucosylation in glycopeptides of monoclonal antibodies

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Keywords:
Glycosylation, glycopeptide, ion trap, MALDI

Novel aspects:
Automatic fragmentation pattern based detection of core fucosylation of N-linked glycopeptides in LC-MS/MS datasets

Abstract:
Fucosylation is a common modification of glycans known to affect glycoprotein confirmation and function. alpha-1,6 fucosylation of the chitobiose core commonly found on N-linked glycans plays an important role in many physiological processes such as signaling pathways and immune response. Involvement of core fucosylation has also been shown in pathological processes like cancer and has been described as biomarker for liver cancer malignity. Antibody-dependent cell-mediated cytotoxicity of IgG has been shown to be crucially mediated by different grades of core fucosylation. Here we describe a fast and reliable approach to unambiguously determine core fucosylation directly on the glycopeptides in tryptic digests by MALDI-TOF/TOF and ESI-IT.

Standard glycoproteins like asialofetuin, horseradish peroxidase and a panel of therapeutic and standard monoclonal antibodies (e.g., MOPC21 from Sigma) were reduced, alkylated and trypsinized. Tryptic peptides and glycopeptides were separated by RP-LC and fractions were analyzed by ESI-IT-MS and MALDI-TOF/TOF-MS in positive ion mode. A dedicated software algorithm for N-linked glycopeptide analysis automatically classified MS/MS spectra taking the specific fragmentation patterns of MALDI and ESI CID into account, clearly distinguishing glycopeptides with or without core fucose. Subsequent protein and glycan database searches provided glycan structures and peptide sequences of the respective glycopeptides.

The typical fragmentation patterns derived from N-linked glycopeptide fragmentation by MALDI and ESI analysis was used to filter glycopeptide spectra from a large number of MS/MS spectra. This allowed determination of both, peptide and the glycan masses of individual glycopeptides and enabled detection of fucosylation at the chitobiose core.

In MALDI-TOF/TOF-MS, the N-acetyl glucosamine (GlucNAc) attached to the peptide undergoes a $^{0,2}X$ cross ring fragmentation resulting in the specific fragmentation pattern ($M_{peptide}$+H) / +83/ +120 Da (Wuhrer et al., 2007) . In core fucosylated glycopeptides an additional fucose distance of Dm/z=146 is found adjacent to the fragment peak from the Y type cleavage (Domon and Costello nomenclature, 1988) .

In ESI-IT, the fragment mass of the peptide including the first GlucNAc of the chitobiose is detected in the majority of cases of N-glycopeptides. In addition to this YY or Y fragment (for core fucosylated or non-fucosylated N-glycans) , the ZZ fragment of the peptide & GlucNAc (mass difference -36) , the Z fragment of peptide & GlucNAc & fucose (+128) and the Y fragment of the peptide & 2 GlucNAc & fucose (+ additional 203) are observed and were used for the detection of core fucosylated N-glycopeptides including determination of the peptide mass.

This information was used to adapt the glycoprotein analysis software by extending lists of fragmentation patterns to distinguish different glycan structures. That capacity enabled us to identify the high glycan micro heterogeneity at HC-Asn294 in murine IgG 1 MOPC21. Core fucosylation was found to be a common modification in more than 50 % of the identified N-glycans. The main glycan in MOPC21 is G 0 F 1 (GlucNAc 4 Man 3 Fuc 1) , followed by the same structure lacking the core fucose (G 0) . Subsequently, core fucosylation classification results were verified by glycan database searches and spectrum matches.

PTu-011
11:10 – 12:20

Identification and structural analysis by chip-based nanoelectrospray mass spectrometry of novel brain-associated chondroitin/dermatan sulfate motifs

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Keywords:
glycosaminglycans ; chondroitin/dermatan sulfate ; chip-based nanoESI ; brain

Novel aspects:
Novel oversulfated CS/DS species from brain were for the first time discovered and characterized using fully automated chip-based nanoESI MS and CID multistage MS.

Abstract:
Brain chondroitin/dermatan sulfate (CS/DS) represent a class of glycosaminglycans involved in many biological processes, such as cell proliferation and recognition, cell adhesion and migration, neurite outgrowth and wound repair. CS chain backbone consists of repetitive disaccharide units containing D-glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) residues, whereas DS is the stereoisomeric variant of CS with varying proportions of L-iduronic acid (IdoA) . Recent studies have shown that proportions of CS/DS disaccharide units in brain change with development/affliction suggesting that CS/DS differing in the degree and profile of sulfation may be involved in the functional diversity of neurons [1,2] . In the last few years, due to the technical advancements in MS instrumentation and the development of efficient sequencing protocols [3-5] , the determination of CS/DS structure in terms of chain length, epimerization, sulfation content, pattern and site (s) became feasible.
In this study, CS/DS chains extracted from 4 weeks old normal mouse brain were released by beta-elimination and digested by chondroitin lyases. The enzymatic hydrolysis mixtures of hybrid CS/DS chains were separated by size exclusion chromatography. Tetra- and hexasaccharide pools were submitted to fully automated chip-nanoelectrospray ionization (nanoESI) mass spectrometry (MS) in negative ion mode. Fully automated chip-based MS was performed on a high capacity ion trap (HCT) mass spectrometer couplet to a NanoMate 400 robot. Multistage MS (MS^n) was carried out by collision-induced dissociation (CID) at variable low energies using helium as the collision gas.
For this assay, to discriminate between domains containing GlcA or IdoA, the whole CS/DS chain was digested with chondroitin AC I lyase and in parallel with chondroitin B lyase. Chondroitin B lyase cleaves the linkage between GalNAc and L-IdoA, while chondroitin AC I lyase cleaves the linkage between GalNAc and D-GlcA irrespective of the sulfation content and distribution along the chain or within the monomer. Screening in the MS^1 mode of tetra- and hexasaccharides resulted after depolymerization with AC I lyase revealed molecular ions assigned to regularly and oversulfated species. CID MS^n applied on tetra- and pentasulfated tetra- and hexasaccharides gave rise to a fragmentation pattern characteristic for 4,5-delta- [GlcA (S) -GalNAc (S) -IdoA (S) -GalNAc (S)] and 4,5-delta- [GlcA (S) -GalNAc (S) -IdoA (S) -GalNAc (S) -IdoA-GalNAc (S)] structures. Chip ESI MS profiling and CID MS^n analysis of the two oligosaccharide fractions obtained by depolymerization of the same CS/DS chain using chondroitin B lyase provided evidence upon the occurrence of mono- and bisulfated dissacharide sequences. CID MS^n experiments provided a fragmentation pattern consistent with an atypical, previously unidentified 4,5-delta- [IdoA (S) -GalNAc (S) -GlcA-GalNAc (S)] and 4,5-delta- [IdoA (S) -GalNAc (S) -GlcA-GalNAc (S) -GlcA-GalNAc (S)] structural motifs. Consequently, due to the high sensitivity and reproducibility of the employed MS method, novel brain-associated CS/DS species exhibiting an unusual location of the sulfate groups at GlcA and IdoA were for the first time discovered and characterized. Obviously, this analytical platform opens new perspectives in glycomics of brain glycosaminoglycans for identification of brain CS/DS domains of irregular sulfation pattern and elucidation of their role in development, maturation, aging and degeneration of the central nervous system.

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PTu-012 Glycobiomarkers for atherothrombosis

13:30 – 14:40

Oliver Ozohanics[1], Lilla Turiak[1], Anita Jeko[1], Angel Puerta[2], Jose Carlos Diez-Masa[2], Mercedes de Frutos[2], Karoly Vekey[1], Laszlo Drahos[1]

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Keywords:
Alpha-1-acid-glycoprotein AGP Glycosylation atherothrombosis atheroslerosis

Novel aspects:
Early diagnosis tool for atherothrombosis by analysing AGP glycoforms (78 % sensitivity and 95 % specificity)

Abstract:
Cardiovascular diseases, like atherothrombosis, are the leading cause of death in the western world. Currently there is no mass diagnostic procedure of atherotrombosis, as Computed Tomography (CT) is costly and not suitable for high throughput, and angiography is invasive and not suited for routine diagnosis.
Proteomics is one of the most dynamically improving research field recently focusing on post-translational modifications (eg. phosphorylation, glycosylation). Glycosylation is immensely important; two typical examples are biopharmaceuticals and FDA accepted biomarkers, most of which are glycoproteins. There is a lack of adequate techniques for determining glycosylation patterns, suitable high-throughput analytical methodologies are just starting to appear. In this presentation we will show a novel high-throughput approach to determine site-specific glycosylation patterns.
The developed workflow is based on LC-MS quantitation of glycopeptides and LC-MS/MS based glycopeptide identification. The key, novel aspect of the workflow presented is automatic spectra evaluation. To identify glycopeptide structures based on tandem mass spectra we have developed the algorithm and software named GlycoMiner. This works automatically (does not require manual control) and has a very low false positive and false negative rate (both 0.1 %). To analyze glycosylation patterns automatically, we have developed another software (GlycoPattern). This works in conjunction with GlycoMiner. Besides quantitation, it is capable of identifying minor glycoforms.
We have applied the developed workflow to analyze the glycosylation pattern of α-1-acid glycoprotein (AGP) samples, isolated from the plasma of healthy individuals and patients suffering from 2 different forms of atherothrombosis (carotid atherosclerosis and abdominal aortic aneurism). Statistical analysis showed significant differences among all three groups (healthy, atherosclerosis and aneurism) based on the glycosylation pattern. Cross-validation showed 78 % sensitivity and 95 % specificity for identifying atherothrombosis (comparing sick vs. healthy groups). The glycosylation pattern of AGP is therefore a very promising biomarker candidate.

Acknowledgement:
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PTu-013
11:10 – 12:20

Top-Down Mass Spectrometric Analysis of Glycosylated and Phosphorylated Proteins

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Keywords:
Top-down, N-linked glycosylation site, phosphorylation site, new sample preparation

Novel aspects:
Intact N-linked glycans were detected by positive mode MALDI-ISD and phosphorylation sites were analyzed using negative mode MALDI-ISD.

Abstract:
Introduction :

Top-down approaches allow for direct analysis of intact proteins using their mass and their fragments for identification and characterization. In contrast to bottom-up techniques, labile PTM's like phosphorylation remain stable in top-down analysis and enable direct modification site determination. MALDI-In-Source-Decay (MALDI-ISD) is a powerful Top-down-Sequencing (TDS) tool and typically provides long N- and C-terminal sequence tags of up to 80 amino acids and more, allowing for the detection of large PTM's like N-linked glycans even at central sites of proteins up to 15 kDa. TDS were applied to standard glycoproteins such as RNAseB, horseradish peroxidase, alpha- and beta-caseins for positive as well as negative ionization mode MALDI-ISD was explored.

Methods :

Proteins (Sigma) were dissolved in water/ 0.1 % TFA at 10-100 pmol/ul, applied to a MALDI sample plate carrying 2 mm hydrophilic anchor sample spots, allowed to dry and rinsed. Matrices for MALDI-TDS were 1,5-diaminonaphtalene (DAN) and super-DHB. They were dissolved in 50 % acetonitrile/water/0.1 % TFA and 1 ul was applied to the rinsed sample spots. Spectra were acquired manually or automatically in positive and negative ion mode. Proteins were identified using ISD signals as virtual precursors and as fragments for MASCOT (Matrix Science, Inc.) MS/MS ions searches. Protein sequences were matched to the spectra including proposed modifications and ranked by a proximity score reflecting how closely the sequences match the experimental data.

Preliminary Data :

This presentation describes a new method for sample preparation, TDS spectrum acquisition and interpretation for the analysis of glycosylated or phosphorylated proteins. The usage of the MALDI sample plates with hydrophilic sample areas allowed for washing the dried, buffer containing samples before matrix application. This led to higher S/N values and extended mass range for TDS analyses. ISD fragmentation series are typically terminated by disulfide bonds and limits the utility of TDS.
Glycosylation : Using DAN as matrix, reduction of the SS-bridges occurred and ISD fragmentation of RNaseB was extended to residue 40 including the glycosylated Asn34. The most abundant high-mannose-type glycans Man 5 and Man 6 were detected. Horseradish peroxidase was prepared the same way and the N-linked glycan structure typical for plants was identified at Asn13. Additionally, the pyro-glutamate at the N-terminus of horseradish peroxidase was found. To our knowledge, this is the first time TDS analysis included the assignment of N-linked glycans.
Phosphorylation : Negatively charged phosphate modifications in -casein limited the N-terminal ISD fragment detection in positive ion mode, only 2 phosphorylation-sites were detected. Negative ion mode, however, enabled the detection of all 5 N-terminally phosphorylated serine residues. In -S 1 -casein, Ser--41 was identified as non-phosphorylated in contrast to uniprot description, S46 and S48 were confirmed as being phosphorylated. -S 2 -Casein carries 5 phosphorylated serine residues near the N-terminus, all of them were confirmed using negative mode ISD. As a result, we propose to use negative ion mode ISD in cases where phosphorylation or other negative charged residues effect the formation of fragment ions in TDS analyses.

PTu-014 Design and Testing of a Miniature Magnetic Sector Mass Spectrometer

13:30 – 14:40

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Keywords:
magnetic-sector ; double trajectory ; miniaturization

Novel aspects:
A small, lightweight, low power consumption instrument has been designed. Including the miniaturization of ion source, high voltage technique, sweep high voltage subsystem, and micro-current amplifier subsystem.

Abstract:
A miniaturized magnetic-sector mass spectrometer has been developed and tested. The instrument was designed which consist of Nier-type thermionic ion source, a 90° magnetic sector-field analyzer with double trajectory, electronic unit and ion detectors. Miniaturization (170 x 165 x 170 mm^3,<4.3 kg) was accomplished by using high-energy-product magnet material (NdFeB alloy) which has an magnetic of 0.65T. Two special trajectories are selected to cover the mass ranges 1-12amu and 6-50amu. The electronic unit consists of sweep high voltage subsystem, and micro-current amplifier subsystem. The sweep high voltage subsystem consist of the sweep high voltage supply and the digital sweep control circuit. The sweep high voltage power supply develops the high voltage level on which the ion source rides. Digital control develops a sweep output and a pedestal output. Together these two output control the sweep high voltage power supply. The instrument was able to attain a mass resolution of$7.7x10^{-5}$ A/Pa, and a mass range up to 50 atomic mass units. Its power consumption was measured to be 15W. All these characteristics make this miniature mass spectrometer suitable for space applications and chemical analysis and also for low-cost analytical instrumentation.

PTu-016 Real-Time Monitoring of a Suzuki Reaction using a Hood-Based Compact Mass Spectrometer

13:30 – 14:40

Daniel Eikel, Jack Henion, Simon Prosser, Nigel Sousou, Collier Lee, Jamey Jones

Advion Inc., Ithaca, New York, USA

Keywords:
reaction monitoring, on-site mass spectrometry

Novel aspects:
Real-time reaction monitoring of a Suzuki reaction is described with a hood-based chemical synthesis- compact LC/MS system.

Abstract:
Medicinal chemists are routinely faced with personally synthesizing over a hundred new chemical entities (NCE's) each year for testing as future pharmaceutical drug candidates. The goal is to prepare a high yield, relatively pure product via an optimized synthetic route. TLC and LC/UV/MS are routinely used to monitor and guide these reaction outcomes. In most cases the LC/UV/MS technology is only available through a central core facility or via shared open access of systems. A preferred alternative is a personal small LC/MS system located in a hood adjacent to the synthetic reaction apparatus. This presentation will describe such an LC/MS system used to monitor and optimize a Suzuki coupling reaction.

A compact single quadrupole mass spectrometer (expression, Advion, Inc.) with dimensions of 11 X 21 X 26 inches was installed in a standard chemical fume hood adjacent to a round-bottom flask chemical synthesis setup. The mass spectrometer was equipped with an atmospheric pressure ionization (API) source coupled with electrospray ionization. A Shimadzu 20 AD binary solvent delivery system employing a 2.1 mm i.d. x 5.0 cm C-18 reversed-phase HPLC column operated in the isocratic mode with 40/60 aqueous acetonitrile containing 0.1% formic acid and maintained at 200 microliters/min. The LC/MS system was used to monitor product formation from a conventional Suzuki coupling reaction to prepare p-aminobiphenyl. Direct injection of diluted crude reaction product mixture for LC/MS analysis was used.

The Suzuki reaction selected in this study consisted of coupling p-aminobromobenzene with phenylboronic acid at room temperature stirred with palladium dichloride and NaOH in ethanol. This reaction prepares p-aminobiphenyl at the 5 mmol level which makes nearly a gram of product in 5 mL of a heterogeneous solution if it goes to completion with a quantitative yield. The para amino group retards this coupling reaction thus providing the opportunity to improve the yield with the application of heat or longer reaction time. The described compact LC/MS system thus may be used to monitor the starting material disappearance vs. the production of the desired product during the course of the reaction. A series of reactions were carried out while systematically varying the reaction temperature and reaction time. At regular 10 min. interval five microliter aliquots were manually removed from the reaction vessel, diluted and mixed in a 5 mL injection solvent mixture of 80/20 water/0.1% acetonitrile and then 5 microliters of this solution injected into the LC/MS system under isocratic conditions. The latter 40/60 aqueous acetonitrile containing 0.1% formic acid mobile phase had been previously optimized to provide a run cycle time of only 2 min. with good separation of each targeted compound. A post-column split of 200/ 1 was provided by RMA valve providing reduced exposure of high concentration compounds direct to the compact mass spectrometer. The typical experimental sequence provided real-time LC/MS results of the reaction progress within one-hour reaction intervals. This allows immediate changes in reaction conditions for the next synthetic run allowing the optimization of this reaction to provide a 90% yield within a half-day period.

PTu-017
11:10 – 12:20

Explosives Trace Detection by Mass Spectrometry: An Automated Particle Sampler for Collecting Explosives Particles Adhering to Passenger's Baggage

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Keywords:
Explosives_Trace_Detection, Mass_Spectrometry, Linear_Ion_Trap, Cyclone_Particle_Concentrator

Novel aspects:
Explosives Trace Detection by Mass Spectrometry : An Automated Particle Sampler for Collecting Explosives Particles Adhering to Passenger's Baggage.

Abstract:

Introduction

The threat of terrorism or criminal bombings has become a serious problem for all countries. To maintain a safe society, detection technologies for hidden explosive devices are in high demand. Two methods are primarily used to detect hidden explosives ; bulk detection, which determines the existence of suspicious objects such as knives, firearms, and explosive devices from their shapes, and trace detection, which detects the presence of explosive contaminants by chemical analysis of vapor or particles from objects. The bulk and trace detection methods have different characteristics, and they complement each other's weak points. Therefore, the combined use of bulk detection and trace detection is effective to improve security at important facilities.

We have already developed explosives trace detectors (ETDs) by mass spectrometry (MS) for airport checkpoints. However, current ETDs require human operations for swab-sampling of contaminants from surface of baggage, which makes 100% inspection high-cost. Therefore, an auto-sampling ETD will dramatically reduce human operations, which can be applied to all baggages with lower cost. In this paper, we report preliminary results of an automated particle baggage screener combined with the MS-based ETD.

Methods

The automated particle sampler consists of a nozzle, a sampling port, and a cyclone-type particle concentrator. The compressed air is emitted from the nozzle to the surface of baggage to be checked. Explosives particles adhering to the baggage are detached by the air jet and introduced into the concentrator through the sampling port.

The explosives particles collected by the sampler are then vaporized by a heating unit that is located below the concentrator. The vaporized explosives molecules are introduced into an atmospheric pressure chemical ionization (APCI) ion source and the ions produced by the ion source are analyzed by a linear ion trap mass spectrometer with wire electrodes (wire-LIT) [1,2] .

Preliminary Data

We tested the prototype of the automated particle sampler using NIST SRM2905 which is trace particulate explosive stimulants developed by NIST [3] . The mg -level of the particle explosive simulants, which included ng-level of 2,4,6-trinitrotoluene (TNT) , were adhered to a baggage and we observed the M^- ion (m/z 227) to detect TNT in a negative APCI mode. The signal of TNT was clearly detected within 5 seconds after the emission of the air jet from the nozzle to the baggage. The collection efficiency of the automated particle sampler, which are defined as the ratio of the amount of the particles collected by the sampler to the amount of the particles initially adhered to the baggage, were 0.4- 1 %.

The sensitivity and throughput of the ETD system is high enough to use it with X-ray bulk detection systems at airport checkpoints. Therefore, we concluded that our newly developed automated particle baggage screener combined by the MS-based ETD would be useful to improve security at important facilities.

This work was supported by "R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society ", Strategic Funds for the Promotion of Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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PTu-019 Radio Frequency Mass Reflectrons with plane Discrete Electrodes

11:10 – 12:20

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Keywords:
discrete planar electrodes, quadrupole fields

Novel aspects:
New method of formation of 2D linear electric fields with discrete planar equipotential electrodes is investigated. This method is based on a linear discrete distribution of the averaged potential.

Abstract:
Modern technologies employ mass spectrometers as an effective means of accurate determination of substance structure. The highest analysis speed belongs to time-of-flight mass spectrometers (TOF MS) . Usually, time-of-flight mass analysis occurs in static fields, which requires high energy homogeneity of injected ions. To overcome this problem time-of-flight mass spectrometers with quadrupole RF fields can be used. Implementation of such devices for time-of-flight sorting requires creation of ion optics with quadrupole RF field extended along the axis of the flight. Hyperbolic electrode systems are not effective here because of large size of electrodes required. Most effectively quadrupole RF fields can be formed by a system of plane electrodes with linear one-dimensional distribution of RF potential. A continuous linear potential distribution, which is difficult to create, is replaced by a discrete linear distribution. Numerical methods were used to study the potential distribution in a flight region. Relative departure of potential distribution was investigated as a function of plane electrodes discrete step size d*y*.
It was found that the value of the departures is proportional to the fifth order of electrode discretion step. The relative departure of potential within the flight region does not exceed 10^{-4} for $dy/L < 0.014$, where L is the length of the analyzer along the axis of the flight. Ion reciprocating oscillations were simulated with a spread in initial energies dW/W=100%, initial coordinates dx/x=0.1, and injection angles ± 5° for the stability parameter 0.01. The relative time defocusing did not exceed 10^{-5}.
Ion-optical systems with a discrete linear potential distribution can be created using inductive and capacitive dividers of RF voltage. Another approach is based on plane discrete equipotential electrodes. Analyzers can be created by a system of plane discrete electrodes with discrete linear distribution of mean potential along one of axis.
Two types of plane discrete electrodes were examined : with different electrical transparency along the *Y* axis and with different distribution of elements along the *Y* axis. In the first case two-dimensional linear electric fields are formed by a system, which consists of paired electrodes, one of which is solid, while the other one, half-transparent, is grounded. The plane half-transparent electrodes are placed along the *Y* axis uniformly with step *dy*. Their width l_i is selected so that the average potential of the element is proportional to its number. In the second case the two-dimensional linear field is formed by a system of paired electrodes, one of which is solid and grounded. The other electrodes represent a set of identical equipotential elements. Fine metallic wires, parallel to the *Z* axis, can be used as elements of these discrete electrodes. The linear distribution of the mean potential within the plane of each discrete electrode is provided by a change in spacing between the wires.
An RF mass reflectron with plane discrete electrodes with variable band width was investigated numerically. Electrode system consists of 40 elements with the following dimensions : x_0=20 mm, y_0=200 mm, dy= 5 mm. Trajectories were simulated for ions injected with a spread in the initial energies W_{max}/W_{min}=3, injection angles +/- 1°, initial coordinates x_{max}/x_{min}=1.5. The obtained accuracy of potential distribution within mass reflectron with plane discrete electrodes corresponds to resolution 1000. Further optimization of electrode system geometry could yield two or three times greater resolution.

PTu-020
13:30 – 14:40

Development of Low Pressure Dielectric Barrier Discharge Ionization Source and Vacuumed Headspace Method for Portable Mass Spectrometer

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Keywords:
Ionization_source, Headspace_sampling, Portable_mass_spectrometer, Illicit_drug_screening

Novel aspects:
High sensitive new ionization source and efficient sample introduction method were developed for portable mass spectrometer.

Abstract:
The abuse of illicit drugs has been a global problem. To prevent the abuse of the illicit drugs, accurate and reliable screenings of those drugs are required. Compared with the conventional immunochemical assay which has been widely used for the onsite screening, mass spectrometers (MSs) are known to produce much more accurate results. The reason why MSs have been not used for the onsite screening despite their potential is that the MSs are not portable due to their size and weight. Therefore, to apply MSs for the screening, we have attempted to develop a portable MS applicable to the on-site initial test. To downsize MS, a small pump must be chosen, which limits the volume of sample gas from the sample introduction part through the ionization source into the mass analyzer, leading to the decrease in the sensitivity. Thus, the challenge in our project was to increase the sensitivity of the portable MS

For the enhancement of the sensitivity, we have developed two new technologies. One was the low pressure dielectric barrier discharge ionization source. Our setup consisted of a glass tube, two electrodes placed outside and inside of such tube and AC source. By applying high AC voltage on electrodes, dielectric barrier discharge was generated inside the glass tube, leading to the low temperature plasma. Sample molecules were ionized during passing through such plasma. We confirmed that our ionization source worked well under 3000 Pa. Compared with the case using the ambient ionization source, it was therefore possible to enlarge the conductance between the ionization source and the mass analyzer, enhancing the efficiency of the ion transfer to that analyzer. Compared with one of ambient ionization, atmospheric pressure chemical ionization, by using our new ionization source, the sensitivity of the portable MS increased more than 10 times.

The other new technology was the vacuumed headspace method. As the headspace of the sample vial is usually connected with a gas chromatograph column or an ambient ionization source, such space is pressurized to transport sample gas. On the other hand, our ionization source was in about 3000 Pa. Thus, it was possible to decrease the pressure of the headspace. The decrease in the pressure of the headspace resulted in the increase in the ratio of the amount of sample gas to that of whole gas in the headspace, because the saturated vapor pressure of sample did not depend on the vial pressure. This indicated that when the flow volume into the ionization source from the sample vial was unchanged between the case with decreasing the vial pressure and that without it, the amount of the sample gas introduced into that source in the former case was larger. When the headspace pressure was decreased to 7000 Pa, the sensitivity of the portable MS increased about 10 times.

In our portable MS, two new technologies described above were incorporated with a miniaturized linear ion trap. For the sensitivity evaluation, we measured one of illicit drugs, methamphetamine, in urine. In the obtained mass spectrum, *m/z* 150 which was assigned to $[M+H]^+$ ion of methamphetamine was detected. The detection limit of our portable MS for methamphetamine was 0.1 ppm in urine, which would be sufficient for its use in the illicit drug screening.

This work was partially supported by "R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society ", Strategic Funds for the Promotion of Science and Technology of the Ministry of Education,Culture, Sports, Science and Technology, the Japanese Government.

PTu-021 Broad range detection in a low field FTICR instrument

11:10 – 12:20

Joel Lemaire[1], Vincent Kromer[1], Gwendoline Jamet[1], Clotilde Le Vot[1], Essyllt Louarn[1], Michel Heninger[1,2], Helene Mestdagh[1]

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Keywords:
Permanent magnet FTICR, Real time, On site detection, Air monitoring

Novel aspects:
Broad range detection in a permanent magnet based FTICR instrument.Combination of different ionization modes.

Abstract:
FTICR instruments based on permanent magnet are well suited for on site measurements. They are easily moveable and provide both a good resolution and a time response of a few seconds. They cannot be very highly miniaturized since a magnetic field higher than about 1 Tesla is necessary for reaching good performances and the number of ions that can be stored is related to the ICR cell dimensions. But compared to FTICR based on supraconducting magnets with weights above 3000 kg and impossible to move while the magnet is on field permanent magnet FTICR with a weight close to 100 kg are small.
Most high field FTICRs are not able to detect low masses, because the cyclotron frequencies become too high for the detection electronic and also because of the limited mass range of the ion guides often used for selection and transmission of the ions.
In our low magnetic field instruments it is possible to cover the range going from m/z 2 to 500 and therefore to detect all gases from hydrogen to high mass volatile organic compounds with a resolution allowing to distinguish compounds having the same nominal mass but different molecular formula.
Analysis of volatile organic compounds is done using chemical ionization. Most of them will be ionized by proton transfer from H_3O^+ precursor ions with little fragmentation so that only one ion will be present on the mass spectrum for each neutral present and the analysis will rely on precise mass identification. Alternative precursors such as O_2^+ or CF_3^+ or negative ions such as OH^-, O^- can be used to ionize alkanes, CFCs or HCFCs. For small molecules such as CO_2, NO, NO_2 and for main compounds of the air matrix electron impact or charge transfer reactions from rare gases can be used.
We will show examples going from the detection of small masses (H_2^+, HD^+, H_3^+, D_2^+, He^+) to large volatile organic compounds. As the resolution is highest for small masses the different hydrogen isotopomers can be distinguished. And the high resolution (>10000 over the whole mass spectrum) allows for the monitoring of complex mixtures with many compounds simultaneouly present.
The excitation conditions have to be adapted in order to be able to detect the ions over a wide frequency range.
We will give examples of reactions involving both hydrogen and volatile compounds of high mass, followed as a function of time in order to determine their kinetics.

PTu-022 Digital Linear Ion Trap in a Portable Mass Spectrometer

13:30 – 14:40

Zhengxu Huang[2], Hui Mu[1], Xiaoyu Meng[1], Junsheng Zhang[1], Lin Tao[1], Gongyu Jiang[1], Li Ding[1], Xu Wu[2], Zhen Zhou[2], Wenjian Sun[1], Zhong Fu[2]

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Keywords:
digital linear ion trap, portable instrument, VOC detection

Novel aspects:
High performance such as resolution and sensitivity was expected to be obtained.

Abstract:
A digital driven ion trap based on ceramic plate structure was used for a portable mass spectrometer for the first time, and it was currently mainly targeted for detecting volatile organic compounds (VOCs) in the setting of environmental monitoring.
In this device, the VOC samples were introduced into the system through a heated PDMS membrane and ionized by photo ionization source in the vacuum condition. The ions were focused by a set of lens into a linear ion trap (LIT) which was fabricated using silver printing process on the surface of ceramic plates. The LIT was driven by digital square wave in frequency scan mode with amplitudes of 100V or 250V (0-p) according to the specific mass range. A diaphragm pump and a turbo pump were used for maintaining the pressure constant at 0.05Pa using helium as carrying gas, cooling gas, and collision gas.
According to the design, a mass range of 15-800 Th can be expected to cover the major environmental VOCs. For the scan speed of 10 to 50 Hz, a peak width of 0.4Th (FWHM) can be expected. MSn can be carried out in the LIT and detailed results will be shown.
By using the digital technology the electronics and its control can be relatively simple and flexible, whereas the ceramic plate method eliminates the need for high precision machining of hyperbolic shape electrodes while maintaining good resolution and sensitivity. The results for testing various VOCs such as BTEX, formaldehyde, acetone at ppb level will be shown to verify the performance of the device, and application data for using the device in a practical setting will also be presented.

PTu-023
11:10 – 12:20

Photo ionisation time-of-flight mass spectrometry as a powerful tool for the on-line analysis of tobacco and wood combustion and pyrolysis

Christian Busch[1], Thorsten Streibel[1,2], Chuan Liu[3], Kevin G McAdam[3], Christian Radischat[1], Ralf Zimmermann[1,2]

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Keywords:
Photo ionisation time-of-flight mass spectrometry, biomass, combustion, pyrolysis, PAH

Novel aspects:
The first hyphenation of a PI-TOFMS and a cigarette smoking simulator as well as a stove was utilised to reveal formation mechanisms and pathways of health relevant compounds during combustion.

Abstract:
First, a coupling between a cigarette smoking simulator and a time-of-flight mass spectrometer was built to enable the sampling and analysis of fresh tobacco smoke under simulated burning conditions which are closely related to the conditions of a real burning cigarette. However, the cigarette smoking simulator also allows cigarettes to be "smoked " apart from normal burning conditions by a flexible control of parameters such as smouldering and puff temperatures as well as combustion rate and puffing volume in order to allow an investigation of the compounds' formation mechanisms and pathways. The first study of the system included the "smoking " of reference cigarettes (here : 3R4F) under nitrogen atmosphere to separate pyrolysis from combustion processes.
The second part addresses a measurement campaign, which was carried out at the Technology and Support Centre (TFZ, Straubing, Germany) , concerning wood combustion in a normal stove under several normal and malfunctioning conditions, which includes the usage of spruce and beech wood as well as the usage of artificially dried and wet wood. The sampling point of the mass spectrometer was located in the raw exhaust pipe. The objective of the study was the correlation of certain burning phases with the formation of several health relevant compounds such as polycyclic aromatic hydrocarbons (PAH) .
Both approaches enable the direct sampling and analysis of almost unaged smoke which is a complex and dynamic matrix. Therefore, time-of-flight mass spectrometry together with photo ionisation (SPI = single photon ionisation ; REMPI = resonance enhanced multiphoton ionisation) was applied to analyse these mixtures on-line with a high time resolution. Both photo ionisation techniques are unable to ionise prominent bulk compounds of combustion emissions such as nitrogen, carbon dioxide or water. In addition, REMPI is highly selective and sensitive for the detection of phenols and PAH.
The two systems demonstrate clear distinctions between the different experimental conditions based on their corresponding mass spectra and further statistical evaluations such as principal component analysis. In the first experimental setup the yield of nearly all compounds decreased while changing the burning atmosphere from inert to oxidative. Other compounds such as benzene and phenol were not significantly influenced by the type of burning atmosphere. The second setup reveals that the maximum compounds' yields can be measured over a few minutes directly after each new load of wood and during the experiments involving the artificially dried and the wet fuel. Partially, a prompt change from pyrolysis to combustion could be observed by monitoring their particular marker compounds.

PTu-024
13:30 – 14:40

Development of a multi-turn time-of-flight mass spectrometer with an atmospheric ionization

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Keywords:
on site real-time analysis

Novel aspects:
API-MULTUM with the particular linear ion trap was developed.

Abstract:
In recent years, on site real-time analysis by a mass spectrometer is required. To achieve on site real-time analysis, the mass spectrometer needs to have the following specialty. First, the instrument needs to be small enough to carry. Second, high mass resolving power is desirable to analysis without any preparation. Last, an atmospheric pressure ionization (API) source such as a direct analysis in real time (DART) is recommended. The size of a miniature multi-turn time-of-flight (TOF) mass spectrometer (MULTUM-S II) is, including vacuum system and high voltage circuit unit, 50.4 cm x 58.4 cm x 27.3 cm and the resolution of it achieved 31,600. Therefore an API-MULTUM might achieve on site real-time analysis.

We had developed the API-MULTUM, which had commercial API ion source. Various samples, for example cold medicine, were analyzed and their resolution achieved 10,000. However, detection limit of this equipment was not enough. It is because the amount of detectable ions is limited by the width of MULTUM's electric sectors when ion packets are accelerated orthogonally. It was estimated that the amount of detectable ions was about ten millions of ions inleted to acceleration region. As a result, it is clearly that traditional orthogonal acceleration method is not befitted for the interface of an API ion source and MULTUM. Therefore we developed a new connection method and produced the equipment consisted of miniature differential pumping chamber, the particular linear ion trap (LIT) , a potential lift and the MULTUM.

The ions generated by DART like ionization using dielectric barrier discharge were inhaled into the equipment. Through three steps differential pumping room, the ions were transported to the LIT by an RF ion guide system. The length from sampling orifice to the entrance of the LIT was less than 150 mm. The LIT consists of plate electrodes that are inserted between the rod electrodes. The shape of the rod electrodes of the LIT was hyperbolic, and their radius of inscribed circle is 5.307 mm. The length of them was 27 mm. While the ions were trapping, their kinetic energy were collisionally reduced by leaked He gas. When the ions were ejected from the LIT, the RF voltage was shut off, since the RF voltage can influence the TOF spectrum. Then, applying a pulse voltage to the plate electrodes, the trapped ions were ejected. When a positive ion packet passed through the rod electrodes, it was accelerated through the potential difference between the center of LIT and the potential-lift whose length was 100 mm. During the passage of the ions through the potential-lift, the voltage on the potential-lift was switched from 2.0 kV to ground potential (0 V) . Their velocities were conserved when the voltage of the potential-lift was changed. The accelerated ions were spatially focused. As a result, they could be efficiently injected the sector electrodes of the MULTUM. The injected ions were separated at high resolution and detected by a micro channel plate.

PTu-025
11:10 – 12:20

Sample Gas Concentration and Pulsed Injection System for a Portable Ion Trap Mass Spectrometer

Hyun Sik Kim[1], Seung Yong Kim[1], Yong Hak Kim[1], Mo Yang[1], Minyoung Youn[1], Yong-Moon Lee[2]

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Keywords:
Sample gas concentration, Pulsed injection, Ion trap mass spectrometer, CNT adsorbent, Field portable mass spectrometer

Novel aspects:
Sample gas concentration to enhance sensitivity up to 100 times, Pulsed injection of sample gas as small as 50 nL, Application in connection with field portable mass spectrometer.

Abstract:
A field portable mass spectrometer [1] designed for detection of trace gases in the air is studied for improvements ; an energy saving feature to cope with the power consumption for vacuum pumping, and the improvements for detection sensitivity. Because the trace gas components only amount as low as ppm level, they are outnumbered by background air molecules. As a possible solution to the problems, a sample gas concentration system coupled with a pulsed sample injection device was considered. Such a system can reduce power load caused by vacuum pumping, and it can removes background air molecules to enhance the sensitivity for trace gases. Based on a number of reports [2] [3] that described the adsorption/desorption characteristics of carbon nano-tube (CNT) , it is possible to enhance the sample gas concentration in the air up to 100 times. Present contribution describes a study for sample gas concentration system that consists CNT adsorbent and a pulsed sample gas injection system. The goals of this work are ; (1) to develop a versatile concentration system for various trace gas species in the air, (2) to control the memory effect under a repeated application for various samples, (3) to develop a pulsed injection of concentrated sample gas for a portable ion trap mass spectrometer. A CNT-metal nano-composite is used as an adsorbent that is controlled by a small vacuum pump and a heater for sample accumulation, evacuation, and desorption processes. As small as 50 nL of concentrated sample gas is possibly injected to the tiny ion trap mass spectrometer [1] . Experimental results with the mass spectrometer are discussed.

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PTu-026
13:30 – 14:40

Various erosion rates of weathered soil surfaces of mountain ridges in Tohoku District, Japan using in-situ cosmogenic nuclides depth profile.

Kazuyo Shiroya[1], Junichi Itoh[1], Yusuke Yokoyama[2], Hiroyuki Matsuzaki[2]

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Keywords:
In-situ cosmogenic nuclides, depth profile, weathered surface erosion rate, Tohoku District Japan

Novel aspects:
Quantitative determination of surface erosion rates using 10Be and 26Al depth profiles

Abstract:
Erosion rates of landforms are important information for quantitatively understanding geomorphological processes. However, it is not easy to quantitatively determine erosion rates because eroded materials are removed from landform surfaces. Recently, multiple-depth profiles of in-situ produced cosmogenic nuclides (e.g., ^{10}Be and ^{26}Al) constitutea powerful tool for directly, accurately, and quantitatively determining erosion rates of weathered soil surfaces of mountain ridges in humid areas (e.g., Shiroya et al., 2010) . In-situ cosmogenic nuclids are produced by interactions with secondary cosmic rays (muons and neutrons) near the surface, which decreases with depth accompanied with attenuation of secondary cosmic rays (e.g., Gosse and Phillips, 2001) .

We present erosion rates of weathered soil surfaces of mountain ridges in Tohoku District (Abukuma and Kitakami areas) , Japan using a depth profile of in-situ produced cosmogenic ^{10}Be and ^{26}Al. Tohoku is a well-known study area for fore-arc tectonics. However, the relationship between landform evolution and factors such as tectonics, climate and weathering has not been quantitatively discussed. Measurements of ^{10}Be and ^{26}Al in quartz are performed by AMS at the University of Tokyo. Results show that the study sites have significantly various erosion rates. This suggests that erosion rates in Tohoku District are not uniform. In this presentation, we will discuss causes of heterogeneous erosion rates in Tohoku District (Abukuma and Kitakami) .

PTu-027
11:10 – 12:20

Estimation of Isotopic ratio of radioactive iodine (I-129/I-131) released from Fukushima Daiichi NPP accident

Yasuto Miyake[1], Hiroyuki Matsuzaki[1], Takeshi Fujiwara[1], Takumi Saito[1], Takeyasu Yamagata[2], Maki Honda[2], Yasuyuki Muramatsu[3]

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Keywords:
Fukushima, iodine, AMS, isotopic ratio, soil

Novel aspects:
The average isotopic ratio derived from Fukushima Daiichi nuclear power plant accident was estimated to be I-129/I-131 = 36.8±9.7 as of March 15, 2011.

Abstract:
On March 2011, an accident caused by a great earthquake occurred at Fukushima Daiichi nuclear power plant (NPP) and radionuclides were discharged. Among them, Iodine-131, which has a short half-life of 8 days, is thought to be hardly detected several months after. It is very important to research how leaked out Iodine-131 was diffused in order to estimate the health impact of radiation at the time of the accident. On the other hand, Iodine-129, which was leaked out and was thought to act chemically-identically as Iodine-131, has an extremely long half-life of 1.57×10^7 years and it can be measured it equally after the accident. By following the trail of Iodine-129, it is considered to be able to estimate the distribution of Iodine-131. For this, it is essential to measure the I-129/I-131 isotopic ratio of Iodine derived from the nuclear power plant.
In this study, Iodine-129 in surface soil within 60 kilometers from Fukushima Daiichi NPP was measured. Soils were collected and Iodine-131 level in soil had already been determined by research team of Nuclear Professional School, School of Engineering, The University of Tokyo. We discuss Iodine-129 derived from the Fukushima Daiichi NPP by considering the I-129/I-131 ratio. Since Iodine-129, which had been leaked out from the nuclear fuel reprocessing plant in Europe, was already transferred to Japan by way of the atmospheric transportation before the accident, it is important to distinguish between Iodine-129 from this accident and from the reprocessing plant. Then, we want to obtain the I-129/I-131 ratio originating in the accident precisely.
As the way of experiment, iodine was extracted from soil and mixed with a carrier and I-129/I-127 ratio was determined by AMS (Accelerator Mass Spectrometry) at MALT (Micro Analysis Laboratory, Tandem accelerator, The University of Tokyo) .Iodine-127 was measured by ICP-MS.
As a result, certain apparent correlation between I-129 and I-131 from soil was observed. Surface deposition amount of I-129 ranges between 11.77 mBq/m^2 and 6057.6mBq/m^2 within the area form 3.6 km to 59.0 km distant from Fukushima Daiichi NPP. Iodine-127 concentration was distributed from 0.21 to 17.41 ppm. The average isotopic ratio was estimated to be $^{129}I/^{131}I$ = 36.8±9.7 as of March 15, 2011.

PTu-028 Depth profile and migration of 129I concentration in soil at Abukuma, Fukushima

13:30 – 14:40

Maki Honda[1], Hiroyuki Matsuzaki[2], Takeyasu Yamagata[1], Yoko Tuchiya[2], Chuichiro Nakano[2], Yuki Matsushi[3], Hisao Nagai[1], Yuji Maejima[4]

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Keywords:
AMS, 129I concentration, Depth profile, Migration, Fukushima

Novel aspects:
In this context, 129I accidentally released from the F 1 NPP accident is the 129I spike into the environment with precisely known source position and time and ideal tracer for this study.

Abstract:

1. Introduction

The long-lived radionuclide ^{129}I ($T_{1/2}=1.57\times10^{7}$ yr, $E_{\beta,Max}=0.194$MeV) is widely distributed in environment. Natural production of ^{129}I occurs by cosmic-ray interactions with Xenon in the atmosphere and by spontaneous fission of ^{238}U in the crust, which in equilibrium with ^{127}I and the $^{129}I/^{127}I$ was estimated to be about 1.5×10^{-12} before 1950 (J.E. Moran et al, 1998) . As human began to utilize the nuclear power, enormously large amount of anthropogenic ^{129}I has entered into the environmental iodine cycles and elevated $^{129}I/^{127}I$ by several orders of magnitude above the 1950 levels.

To evaluate the migration of iodine in the soil, long-lived radionuclide ^{129}I is very useful tracer. The anthropogenic ^{129}I has been produced only recent years (after 1950's) so that its distribution in the environment has not yet reach equilibrium at all. Thus we can investigate the global dynamics of iodine by observing the distribution of anthropogenic ^{129}I. In this context, ^{129}I accidentally released from the Fukushima Daiichi nuclear power plant (F 1 NPP) accident is the ^{129}I spike into the environment with precisely known source position and time and ideal tracer for this study.

This paper reports the analytical results for $^{129}I/^{127}I$ ratio depth profile in the soil collected from the area influenced by the F 1 NPP accident.

2. Experimental

In this study, three 30cm-soil cores were analyzed, of which sample codes are ABK2, ABK 3 and ABK12. ABK 2 and ABK 3 were collected in the Abukuma mountain region in Fukushima prefecture about 30km distant from F 1 NPP in the South West direction. These were collected at almost the same position in a forest but before and after the accident so that we can observe the influence from the accident directly by comparing ABK 2 and ABK3. ABK12 was collected at the crop field 20km apart from the F 1 NPP after the accident.

The soil sample were first dried by oven. Then homogenized well. An amount of about 0.5g was mixed with V_2O_5 in a ceramic boat and placed in a quartz tube. The sample is then heated at 1000&#8451 ; under a flow of oxygen gas. The evaporated iodine is collected with a trap solution (2 % TMAH solution) . (Here, an aliquot was separated for ICP-MS for the determination of the stable iodine concentration.) Iodine in the trap solution with 2 mg iodine carrier is purified by a sequential solvent extraction and back extraction and a finally extracted as AgI precipitation. Extracted AgI was dried well and pressed into a cathode of AMS system. $^{129}I/^{127}I$ was measured at MALT-AMS system, The University of Tokyo.

3. Results and Discussion

At the depth of 1.5cm, $^{129}I/^{127}I$ ratio in ABK 3 (after accident) was five times higher than ABK 2 (before accident) , which should be the accumulation of ^{129}I ejected by the F 1 NPP. At the other depths, almost identical $^{129}I/^{127}I$ profiles were observed. The maximum $^{129}I/^{127}I$ ratio in ABK 3 (1.29×10^{-7}) was much higher than the surface ratios reported before the accident, at most 3×10^{-8}.

ABK12 was collected from a crop field and considered to be well mixed. This is supported by the observation that the depth profile of ^{127}I concentration was constant and lower than the surface layer of forest. $^{129}I/^{127}I$ decreases rapidly from the surface to at 9 cm depth and becomes constant below it. We considered this enhancement in the top 9 cm layer was the direct accumulation from the F 1 NPP after the accident. Considering constant level at lower layer ($^{129}I/^{127}I=8.24\times10^{-9}$) as a background, ^{129}I inventory was estimated as 3.41×10^{13} atoms m^{-2} (47.7mBq m^{-2}) .

PTu-029 Progresses in sample treatment for BAMS analysis

11:10 – 12:20

Minyoung Youn[1], Seung Yong Kim[1], Yong Hak Kim Kim[1], Hyun Sik Kim[1], Jae-Gook Shin[2], Ji Hong Son[2], Soo Kyung Bae[2], Hyun Sic Chae[3]

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Keywords:
BAMS, Sample treatment

Novel aspects:
Improved efficiency in the treatment time. Measures elemental density of carbon component in original sample.

Abstract:
Biomedical application of AMS (BAMS) is widely used in new drug development as well as in researches. Owing to the sensitivity of AMS analysis, BAMS application fields expand. The status being as useful as it is, additional modification and improvement may contribute to the BAMS usefulness : the time taken for pre-treatment of samples needs to become as short as possible. A conventional pre-treatment of a sample for BAMS analysis is performed through three steps ; dehydration, combustion and reduction. In this study, we merged the combustion and the reduction to happen in series. We used an element analyzer (E/A) to combust the dehydrated samples. The dump gas of the element analyzer was put to an additional column to totally discriminate SO_2, NO_2, and H_2O. Residual CO_2 gas was introduced to a reduction reactor that was selected using a selection switch valve. Total 5 reaction chambers were constructed to perform graphite reduction in parallel. Overall treatment time taken for combustion and reduction ranges 2~4 hours. In addition to the progress in time-saving feature, another advantage found in the course is ; it became possible to deduce the density in the original sample by combining the E/A analysis to BAMS analysis ; the carbon concentration in the sample can be measured using E/A, while the drug concentration in the dehydrated sample is measured through BAMS analysis. In this contribution, details of the device design, the construction and the experiment result are presented.

PTu-030 Soft-sputtering of protein molecules using various gas cluster ion beams

13:30 – 14:40

Kousuke Moritani, Kosuke Goto, Issei Ihara, Norio Inui, Kozo Mochiji

University of Hyogo

Keywords:
Soft-sputtering, SIMS, gas cluster ion beam, Ar cluster SIMS

Novel aspects:
The novel gas cluster ion projectiles besides Ar cluster were applied to SIMS for biomolecule measurements. The effect of cluster species on soft-sputtering will be discussed.

Abstract:
A gas cluster ion beam (GCIB) provides an extremely-low energy projectile compared to conventional polyatomic projectiles for SIMS. Because the kinetic energy of the cluster is shared by the constituent atoms, individual atoms have small kinetic energy in a cluster composed of numerous atoms. Therefore, the damage of organic molecules can be substantially suppressed. Additionally, numerous atoms simultaneously collide. Thus, the dense energy is deposited on the surface, causing the soft-sputtering of the protein molecules.

We have investigated the damage formation on highly orientated pyrolytic graphite (HOPG) and the sputtering of DNA molecules adsorbed on HOPG by Ar-GCIB. [1] This study has suggested that a kinetic energy per constituent atom of cluster (E_{atom}) was the most important variable to the damage formation on the sample. Moreover, it has been indicated that adjusting E_{atom} of the GCIB can suppress damage on the sample. Motivated by these experimental results, we have developed a size-selected gas-cluster SIMS apparatus [2,3], where E_{atom} can be controlled from several tens eV to below 1 eV by selecting the cluster size and the acceleration voltage of the gas-cluster ion. On applying this instrumentation to several protein and peptide molecules, we demonstrated that the fragmentation is substantially suppressed when E_{atom} was decreased below ~5 eV and intact ions of some protein molecules, for example, insulin (molecular weight : 5,808) and chymotripsin (molecular weight : ~25,000), were detected without using any matrix. [4,5] We have focused on the dependence of the emission of intact ions and fragment ions on the incident direction of the cluster ions as well as accumulated dosage of the cluster ions. [6] The bombardment at a lower angle of incidence significantly enhanced the emission of intact ions. However, the yields of intact ion should be enhanced still more for practical use.

Although there has been much progress in the use of Ar cluster ions as projectiles for the detection of intact ions of biomolecules, details of the role of the cluster ions in soft-sputtering and ionization, remain unknown. Recently, it is reported that insulin molecules were ionized and emitted to vacuum without matrix by neutral SO_2 cluster collision. [7] This result suggests that the chemical effect in cluster collision plays a role in soft-ionization of protein in addition to the physical sputtering processes. We have investigated the soft-sputtering of protein molecules by Ar GCIB and some other gas cluster ion beam. In this presentation, results of the biomolecule measurement in SIMS by various gas cluster ion projectiles will be presented.

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PTu-031
11:10 – 12:20

Sensitive Ionization of Non-Volatile Analytes Using Multiply Charged Primary Ions in Desorption Electrospray Ionization Mass Spectrometry

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Keywords:
DESI, proteins, non-volatile analytes, ambient mass spectrometry, explosives

Novel aspects:
This is a novel and deformed DESI method with higher sensitivity for the non-volatile analytes.

Abstract:
Desorption electrospray ionization (DESI) is the first and the most popular ambient ionization technique and it can analysis complex sample without sample pretreatment. DESI-MS has been successfully applied in numerous fields. However, for many applications, especially for trace analysis, it is of interest to improve the sensitivity of DESI-MS.

In traditional mass spectrometry DESI, a mixture methanol/water/HAc is usually used to generate the primary ions. In this article, dilute protein solutions were electrosprayed in DESI method to create multiply charged primary ions for the desorption ionization of trace analytes on various surfaces (e.g., filter paper, glass, Al-foil) without any sample pretreatment. The ionic compounds were detected by the LTQ XL mass spectrometer.

Compared with the methanol/water/acetic acid (49 : 49 : 2, v/v/v) solution, protein solutions significantly increased the signal levels of non-volatile compounds such as benzoic acid, TNT, o-toluidine, peptide and insulin. For all the analytes tested, the limit of detection (LOD) was reduced to about half of the original values which were obtained using traditional DESI. The results showed that the signal enhancement is highly correlated with the molecular weight of the proteins and the selected solid surfaces.

Our provided simple and novel DESI method is a universal and useful strategy for rapid and sensitive detection of trace amounts of strongly-bound and/or nonvolatile analytes, including explosives, peptides, and proteins. The results hit that it can further improve the sensitivity by selecting larger protein and appropriate solid surfaces.

PTu-032
13:30 – 14:40

The Gas Phase Mechanism of 2-Furaldehyde Formation from Pentose Sugars: A Mass Spectrometric and Theoretical Study

Federico Pepi[1], Andreina Ricci[2], Simona Piccolella[2], Stefania Garzoli[1], Rino Ragno[1], Alexandros Patsilinakos[1], Pierluigi Giacomello[1]

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Keywords:
Biomass, Pentoses, Dehydration Mechanism, Energy Resolved Mass Spectra, Triple Quadrupole

Novel aspects:
The gas phase mechanism of pentose sugars dehydration leading to 2-furaldehyde was clarified by tandem mass spectrometry and theoretical calculations.

Abstract:
Mass spectrometric techniques offer the possibility to investigate the gas phase mechanism of fundamental reactions in solution allowing to clarify controversial mechanistic hypothesis and to propose simplified and generalised reaction models not influenced by the precence of the condensed medium and of counter ions.
It is generally known in the carbohydrates chemistry that 2-furaldheyde is the main product of the thermal acid catalyzed dehydration of pentoses in solution. Taking into account the Kyoto protocol and the need of reducing society dependence on imported crude oil, the scientific research has been recently focussed toward this process as an important chemical transformation of biomass for the production of sustainable energy and useful chemicals [1-2] .
The acid catalysed decomposition of D-xylose leading to 2-furaldehyde has been investigated in a number of studies dating back to the first years of the XIX century, but its mechanism is still controversial.
In this work we report the first study of the gas-phase decomposition of protonated pentoses (xylose, ribose and arabinose) performed by Electrospray Triple Quadrupole Mass Spectrometry.
Gaseous ionic species of interest, generated in the ESI source and structurally characterized by CAD mass spectrometry, were allowed to undergo collisionally induced dehydration into the triple quadrupole collision cell. Energy threshold values of the consecutive dehydration steps were derived by the energy resolved mass spectra of the ionic intermediates and were compared with dehydration energy barriers obtained by theoretical calculations.
The picture emerging from experimental and theoretical data allow the mechanism of protonated pentoses dehydration in the gas-phase to be identified and a reasonable new hypothesis for the reaction in solution to be proposed.

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PTu-033

11:10 – 12:20

SEPARATION AND MEASUREMENT OF URANIUM,PLUTONIUM AND NEODYMIUM IN SIMULATE SPENT NUCLEAR FUELS BY EXTRACTIONCHROMATOGRAPHY WITH ISOTOPE DILIUTION MASS SPECTROMETRY

Jinying Li[1,2,3], Guoping Xiao[2,3], Lei Shi[1,2,3]

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Keywords:

MEASUREMENT OF URANIUM,PLUTONIUM AND NEODYMIUM ; SIMULATE SPENT NUCLEAR FUELS ; EXTRACTIONCHROMATOGRAPHY WITH ISOTOPE DILIUTION MASS SPECTROMETRY ;

Novel aspects:

Using 144Nd as isotope spike, coupled Eichrom column methodologies have been employed to measure neodymium in simulate nuclear fuel solution by ID-MC-ICP-MS. Uncertainty of concentration measurement for neodymium is 1.5%.

Abstract:

In this work, the concentration of uranium, plutonium and neodymium in simulate spent nuclear fuel resulting from nuclear fission products was precisely determined by means of ID-TIMS and ID-MC-ICP-MS respectively.

PTu-034 When was gold deposited ?

13:30 – 14:40

Nobuo Takaoka[1,6], Nobutaka Shimada[2], Masako Shima[3], Tomoki Nakamura[4,7], Keisuke Nagao[5], Hitoshi Sagawa[6,8], Ryuji Okazaki[4], Kazuhiko Shimada[4]

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Keywords:
double-beta-decay of Te-130, Te-Xe age determination, hydrothermal gold deposit

Novel aspects:
The Te-Xe dating is a new technique to measure ages of minerals formed through hydrothermal events.

Abstract:
^{130}Te decays to ^{130}Xe with simultaneous emission of two electrons. The decay probability is extremely small : in half-life, as long as 50 billion times the age of universe (13.7 billion years) . Based on the newly determined double-beta-decay half-life of ^{130}Te ($7.0 \pm 0.9_{stat} \pm 1.1_{syst}$) $\times 10^{20}$ yr, we have measured the direct mineralization ages on tellurium-bearing minerals from some hydrothermal gold deposits by the ^{130}Te-^{130}Xe method. The results are as follows : Calaverite ($AuTe_2$) shows the age of 21.9±2.3 million years (Ma) from the epithermal Au-Te vein, Cripple Creek mining district, USA ; Hessite-petzite (Ag_2Te - Ag_3AuTe_2) 34.9±5.4 Ma from the epithermal Au-Te vein, Cash mine, Boulder County, Colorado, USA ; Altaite (PbTe) 1,040±80 Ma from the Au-Te quartz-carbonate vein of the Mattagami Lake mine, Matagami, Quebec, Canada ; Tellurobismuthite-tetradymite (Bi_2Te_3 - Bi_2Te_2S) 9.4±1.2 Ma from Bi-Te quartz vein, Tsushima Island, Nagasaki Prefecture, Japan ; Tellurobismuthite (Bi_2Te_3) 81.4±2.6 Ma from the hydrothermal Au-Te vein, Oya mine, Miyagi Prefecture, Japan ; Tellurobismuthite (Bi_2Te_3) 85±18 Ma from the Au-Te quartz vein, Suwa mine, Ibaraki Prefecture, Japan ; Native tellurium (Te) 2 ± 1 Ma from the epithermal Au-Te quartz vein, Rendaiji mine, Shizuoka Prefecture, Japan. These ages are obviously younger than previously reported mineralization ages by other dating methods such as K-Ar, Ar-Ar or Pb-Pb methods. For example, the age of altaite (PbTe) from the Mattagami Lake mine, Matagami, Quebec, Canada is younger by ca. 1,500 Ma than the ages determined from Pb-Pb data for host rocks. This suggests that activities of geothermal fluids that contained gold and other rare metals such as Te occurred long after the formation of the Archean crust of the region (ca. 2,600 to 2,700 Ma) , or that the altaite vein deposited at the Archean magmatic activities was heated to blocking temperature and reset the isotopic system for ^{130}Xe retention.

PTu-035
11:10 – 12:20

Surface Desorption Atmospheric Pressure Chemical Ionization (DAPCI) Source based on Graphite needle

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East China Institute of Technology,Nanchang,China

Keywords:
DAPCI Graphite needle

Novel aspects:
Garphite needle was used as a discharge needle and an adsorption material

Abstract:
A kind of green ionization source-Surface desorption atmospheric pressure chemical ionization source based on graphite needle which can perform rapid and direct analysis of trace compounds in complex matrices was developed by East China Institute of Technology funded by the National Natural Science Foundation Science Foundation.
Graphite can be used as a discharge needle and an adsorption material. On the base of this, we debug and characterize this intelligent ionzation using representative samples such as explosives, aromatic amines in different substrate surface, then we establish a new intelligent direct chemical ionization source which can directly analyze the trace components in complex matices.
Combining with mechanical design, electronic information processing, process engineering, computer technology, automatic control and many other technologies, we successfully developed an intelligent surface desorption atmospheric pressure chemical ionization source[1-3],which coupled to the linear ion trap mass spectrometer, so as to achieve the function of Man-machine dialogue.
The novel ionization source can be coupled with conventional mass spectrometry with high sensitivity, high specificity and the method can perform direct detection to powder, electrocuprol, suspending liquid, aerosols, especially vivo, real-time, on-line detection and is applied on the rapid detection of various trace compounds in different surface, such as：food, drugs, explosive, textile, leather, plastics. Therefore, it shows promising applications such as industry, agriculture, environmental monitoring, public security and other areas of regarding people's livelihood.

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PTu-036
13:30 – 14:40

Rapid 90Sr detection by MALDI-TOF-MS with a new matrix, dipicolinic acid obtained from an eastern Japan-traditional food, Natto.

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National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan

Keywords:
strontium, isotope, MALDI, matrix, dipicolinic acid

Novel aspects:
MALDI-tof-ms analysis of radioactive strontium (90Sr) itself and its matrix, dipicolinic acid, are new. This method is much faster than the conventional radioanalytical analysis and practical.

Abstract:
Since the nuclear accident in Tokyo Electronic Power Company's Fukushima No.1 nuclear power plant, measurement of the amount of radioactive materials have been quotidian concern not only for the people inside the disaster area but also for all the people in Japan. Initially, the acute effect of ^{131}I (half-life is 8 days) was worried. Still now remains the fear of the effect on a human body caused by radioactive materials with longer lifetimes, ^{134}Cs (2 years), ^{137}Cs (30 years) and ^{90}Sr (28.7 years) contained especially in foods. In case of gamma-emitting nuclide, ^{134}Cs and ^{137}Cs, energy-resolved gamma-ray measurement allows us a simple preparation method for variant analytes, e.g. foods, just cutting and putting into a regular package. On the other hand for a beta-emitting nuclide, ^{90}Sr, conventional quantitative analysis takes long time (~3 weeks). Broad line width of an energy peak for the beta-emitting nuclide has prevented an energy-resolved direct measurement for ^{90}Sr, which forces us a depthful preparation process to prepare pure ^{90}Sr from each analyte.

Based on the circumstance, we developed an entirely new and rapid method for analyzing the radioactive ^{90}Sr by a commercial MALDI-tof-mass spectrometer. As a new matrix, dipicolinic acid (2,6-pyridine dicarboxylate), was introduced. Dipicolinic acid is contained in a Japanese traditional food, *Natto*. Sumi et al.[1] had presented that about 1 gram of dipicolinic acid can be obtained from 1 litter culture medium of *Bacillus subtilis Natto*. And dipicolinic acid has high affinity to Sr ion. Furthermore, the molecular structure of the dipicolinic acid was similar to that of 3-hydroxypicolinic acid, a matrix for nucleic acid measurement in negative-mode, which let us predict that the dipicolinic acid is ablated by the N_2 laser equipped in the conventional MALDI-tof-mass instrument. Thus, we attempted to use it as a matrix for MALDI-tof-mass analysis of the Sr ion. In this presentation, we will introduce a set of basic experiments to show the efficiency of the MALDI-tof-ms Sr detection using a combination of a stable isotope, ^{88}Sr and a new matrix, dipicolinic acid under the condition containing impurity ions, such as K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, $^{89}Y^{3+}$ and $^{90}Zr^{4+}$.

For MALDI-tof-mass experiments, Shimadzu AXIMA CFR+ was used. Before the experiment, a new stainless sample plate was washed with 1 % formic acid, methanol, acetone, and distilled water, following the recommendation in the instrument manual. Next, the sample plate was cleaned by UV cleaner (UV DRY STRIPPER / CLEANER MODEL UV-1) to decrease the organic impurities observed in the m/z region (85-95) for Sr analysis. The way for the sample preparation was simple. A sample solution containing ^{88}Sr and additional impurity ions, and a matrix solution including dipicolinic acid was mixed and spotted on the MALDI-tof-ms sample plate and dried. Measurement was performed in reflectron-mode.

The results were as follows.

1. Using the dipicolinic acid as a matrix, stable ^{86}Sr, ^{87}Sr and ^{88}Sr peaks were observed depending on its abundance ratio, even with the other impurity ions in the sample solution.
2. All the alkali soil metals ions (Sr^{2+}, Ca^{2+}, Mg^{2+} and Ba^{2+}) are bound to the dipicolinic acid and observed in the mass spectra as singly-charged ions.
3. The other ions like $^{89}Y^{3+}$ and $^{90}Zr^{4+}$ were not observed at all in the reflectron spectra. It is presumably because those two ions were not selected by the matrix, dipicolinic acid.

These results show that the combination of the ion-selection with the matrix molecule, dipicolinic acid, at the stage of the ionization, and the mass-selection by the mass spectrometer realizes the rapid and practical analysis of radioacrive ^{90}Sr.

[1] Japanese patent, application No. 2006-61039 (P2006-61039A)

PTu-038
13:30 – 14:40

Spatially Resolved Analysis of Uranium and Plutonium Isotope Ratios in Environmental Samples by Laser Ablation MC-ICP-MS

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Keywords:
Radioactive Contamination, Isotope Analysis, Mass Spectrometry

Novel aspects:
Assessment of variation of U and Pu isotopic composition in environmental micro-samples that have been contaminated with spent reactor fuels with different burn-up grades

Abstract:
Monitoring environmental contaminations containing actinides is of special interest for the assessment of hazards for humans, including radiation hazards, and the impact on flora and fauna.
This presentation discusses the application of a multi collector inductively coupled plasma mass spectrometer (MC-ICP-MS) that is equipped with three ion-counting multipliers and coupled to a laser ablation system (LA) for the rapid and sensitive determination of $^{235}U/^{238}U$, $^{236}U/^{238}U$, $^{240}Pu/^{239}Pu$ and $^{242}Pu/^{239}Pu$ isotope ratios in micro-samples collected in an area that had been contaminated with spent reactor fuel in the vicinity of the Chernobyl Nuclear Power Plant (NPP) . Micro-samples with dimensions ranging from ca. 0.1 mm to 1 mm and with surface alpha activities of 3-38 mBq were first identified using nuclear track radiography. U and Pu isotope ratios were then measured sequentially for the same micro-sample by LA-MC-ICP-MS. The application of a zoom ion optic for aligning the ion beams into the ion counters allows fast switching between different isotope systems, which enables all of the above mentioned isotope ratios to be measured for the same micro-sample within a total analysis time of 15-20 min (excluding MC-IC-PMS optimization and calibration) .
A significant variation in uranium and plutonium isotope ratios in micro-samples was observed. $^{242}Pu/^{240}Pu$ and $^{240}Pu/^{239}Pu$ isotope ratios showed a correlation, ranging from 0.007 (2) -0.047 (8) and 0.183 (13) -0.577 (40) for $^{242}Pu/^{240}Pu$ and $^{240}Pu/^{239}Pu$, whereas $^{235}U/^{238}U$ and $^{236}U/^{238}U$ ratios correlated inversely. The heterogeneity of the U and Pu isotopic composition of the individual micro-samples is attributed to the different burn-up grades of uranium in the fuel rods from which the micro-samples originated. In the Chernobyl RBMK-1000 reactor spent fuel was gradually replaced with freshly enriched U during operation, which resulted in different irradiation histories and thus in varying burn-up grades of the fuel assemblies over the reactor core at the time of the accident. Higher fuel burn-up results in generation of more ^{236}U and Pu isotopes and therefore in higher $^{236}U/^{238}U$, $^{242}Pu/^{240}Pu$ and $^{240}Pu/^{239}Pu$ isotope ratios. The measured Pu isotopic signatures were compared to the Pu isotopic composition distribution that is typical for a RBMK-1000 reactor with initial ^{235}U enrichment of about 2 %. A comparison of the measured Pu isotope ratios in Chernobyl soils with Pu isotope ratios in RBMK spent-fuel yielded a good consistency of burn-up grades ranging from about 5 to 12 MWd/kg (U) .

PTu-039
11:10 – 12:20

MALDI MS analysis of N-glycan structures of a cell adhesion molecule, CADM1, in various cancer cells

Tomoko Maruyama[1], Mika Sakurai-Yageta[1], Kaoru Kaneshiro[2], Sadanori Skiya[2], Shinichi Iwamoto[2], Koichi Tanaka[2], Yoshinori Murakami[1]

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Keywords:
N-glycan, MALDI-MS, 3-AQ-labeling, cancer, cell adhesion

Novel aspects:
Different N-glycan structures of a tumor related cell adhesion molecule, CADM1, was identified for the first time in various cancer cells with high sensitivity by 3-AQ-labeling and MALDI MS analysis.

Abstract:
[Introduction] CADM1 (Cell adhesion molecule 1) is a membrane glycoprotein whose expression is frequently down-regulated in the progression of various cancers derived from epithelial cells, including the lung, kidney and breast (1). On the other hand, CADM1 is overexpressed in adult T-cell leukemia (ATL) and small cell lung cancer (SCLC), and promotes their invasion. The molecular weight of CADM1 was different in cancer cells possibly by tissue-specific alternative splicing and/or by different glycosylation. In the extracellular domain (EC) of CADM1, 6 predicted *N*-glycosylation sites are present in 3 immunogloblin (Ig)-like loops (N1-N6). Here, we analyzed the structures of *N*-glycans of CADM1 expressed in epithelial cells as well as those in cancer cells such as renal cell cancers (RCC), ATL and SCLC using glycopeptides and 3-AQ-labeled *N*-glycans by MALDI mass spectrometry.
[Method] CADM1-EC fragment was expressed in HEK293 cells as a fusion protein with Fc domain of mouse IgG (CADM1-EC-Fc) and the secreted protein was purified using protein A beads. CADM1 expressed in various cancer cells were purified by immunoprecipitation using a specific antibody against the cytoplasmic domain of CADM1 and separated by SDS-PAGE. To obtain glycopeptides, in-gel trypsin digestion of CADM1-EC-Fc was then performed and the digested polypeptides were dissolved in a liquid matrix, 3-AQ/CHCA. For 3-AQ-labeling of *N*-glycans, CADM1-EC-Fc or immunoprecipitated CADM1 was in-gel digested with PNGaseF and released *N*-glycans were labeled with 3-AQ/CHCA (2). The mass spectra were acquired using MALDI-DIT or MALDI-QIT-TOF (SHIMADZU Corp.).
[Results] We initially performed MADLI-DIT analysis of desialylated CADM1-EC-Fc glycopeptides, and identified tetra-antennary complex type of *N*-glycans at N2, N3, N5 and N6 by MS and MS^2. By 3-AQ-labeled *N*-glycan profiling, the same structure with a sialic acid was mainly detected in *N*-glycans in CADM1-EC-Fc. The *N*-glycan profiling of CADM1 in RCC, ATL and SCLC cells suggests that cancer cells displayed heterogeneity in *N*-glycans of CADM1, and that the structures of *N*-glycans predominantly seen in ATL/SCLC were distinct from that in epithelial cells. Different glycosylation patterns of CADM1 in epithelial and ATL/SCLC cells could be one of the molecular bases of distinct roles of CADM1 in tumor suppression in epitheila and in cell invasion in ATL/SCLC, and could provide possible molecular targets for the diagnosis and treatment of these malignant diseases.

Reference

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PTu-040
13:30 – 14:40

Isolation and identification of glycopeptides with isomeric glycans by lectin-column chromatography and negative-ion MALDI-QIT-TOF MSn

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Keywords:
Glycopeptide, MALDI, CID, Negative-ion, Pyrene derivatization

Novel aspects:
We proposed the useful method for analysis of glycan isomers on peptides, by combination of the lectin-column chromatography and the on-plate PDAM derivatization followed by negative-ion MALDI-MSn.

Abstract:
Glycosylation is the most common post-translational modifications of proteins, which may have profound effects on protein structure and function. Glycoproteins are heterogeneous with respect to their glycan structures. Various glycans can be attached to the same position on the protein backbone. A distinct glycan structure depends on a particular site and it is referred to as site-specific glycosylation. In fact, analysis of glycans is more complicated and harder than genomics or proteomics because glycans have branched structures and the diversity of monosaccharides. In addition to the structural diversity, glycans on the proteins show dynamic changes in the course of development, differentiation, metabolic changes, malignancy, inflammation, or infection. The structural changes should be associated with functional changes of the glycoproteins. Therefore, we have to know the information of the alteration of glycan structure and the site of glycosylation on the protein as well as the protein. For this reason, we have investigated glycopeptides without releasing glycans.

Mass spectrometry (MS) is a powerful tool for glycoproteomics. Generally, however, glycoprotein analysis using MS is not easy compared to proteins. There are some limitations that the hydrophilic nature of glycopeptides is disadvantageous for ionization, and the amount of each glycopeptide is smaller than nonglycosylated peptides because of heterogeneity. A step of enrichment of glycopeptides is necessary. To discriminate glycan isomers, and also to concentrate the specific glycopeptides, lectin-column chromatography is helpful for MS analysis of glycan structures on the proteins. Lectins are proteins, which specifically recognize glycan structures. Therefore, the specific binding of lectins to glycan structures is used to characterize glycoconjugates.

Regarding sensitivity, conventional proteomics was difficult to detect altered glycosylation on proteins. Recently, our group has established an on-plate pyrene derivatization method using 1-pyrenyldiazomethane (PDAM) for highly sensitive matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS^n) of glycopeptides in amounts of less than 100 fmol (J. Amano *et al., Anal. Chem.,* **82**, 8738-8743 (2010)). Collision-induced dissociation (CID) is effective for glycans structural determination. In glycans analysis, negative-ion MS^n spectra derived from such ions contain many fragment types (B and Y, C and Z, and A) and therefore are more informative than the positive-ion MS^n spectra derived from such ions usually consist mainly of B and Y fragment types. In particular the internal ions (D- and E-type ions) provided useful information about the branching patterns of glycans.

In this study, we attempted to discriminate isomers of tri-antennary *N*-glycopeptides (NA3 peptides) obtained from alpha1-acid glycoprotein or alpha1-antichymotrypsin. First, NA3 peptides were separated from other glycopeptides and enriched by a *Phaseolus vulgaris* agglutinin (PHA)-L_4-agarose column. PHA-L_4 is a lectin that recognizes beta-6,2-GlcNAc branch of *N*-glycan and the glycan can be separated from other isomers. Next, the enriched NA3 peptides were treated with the on-plate PDAM derivatization for highly sensitive MALDI-MS^n analysis. The negative-ion CID of PDAM-derivatized NA3 peptides showed characteristic spectral patterns. In addition, isomeric NA3 on peptides could be discriminated based on their the negative-ion MS^3 spectra by selecting A ion as a precursor ion. The NA3 containing beta-2,4,2-GlcNAc branch gave an abundant E-type ion (*m/z* 831). The structure-depend fragment ion helped to identify NA3 isomer. These data made it possible to discriminate beta-2,4,2- and beta-6,2,2-GlcNAc branch of NA3 on the specific glycosylation site.

Thus, we proposed the useful method for analysis of glycan isomers on peptides, by combination of the lectin-column chromatography and the on-plate PDAM derivatization followed by negative-ion MALDI-MS^n.

PTu-041
11:10 – 12:20

A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MSn system

Shuuichi Nakaya, Yuzo Yamazaki

Shimadzu Corporation, Kyoto, Japan

Keywords:
MALDI, MSn, Glycomics, Glycan

Novel aspects:
We provide a workflow for glycomics researches using offline LC-MALDI and improved software for identifying isobaric isoforms of glycans.

Abstract:
Nowadays, analysis of glycosylation is one of the indispensable approaches for a development of antibody-drug and biomarker discovery, because a large number of proteins in eukaryotes are glycosylated and they play various roles in physiological function like a molecular recognizing. Therefore, a well-established workflow for characterization of glycosylation has been a one of the growing demands. The author and co-workers has reported a system for identification of glycan structures using an observational MS^n spectral library obtained by MALDI-QIT-TOF MS[1),2)]. We demonstrates a practical workflow combined with the spectral library and off-line separation system for the glycan structure characterization.

In this study, glycans released from proteins with N-glycosidase were pyridylaminated and purified by cellulose and graphite carbon tip. A small aliquot of a purified glycan mixture was subjected to MALDI MS analysis directly and a list of candidate precursor ions was generated by the library search system, which is revised to correspond to various chemical labeling methods (Accurate Glycan Analyzer 2 ; AGA 2 / Shimadzu Corp.) . The remaining sample was provided to a nano HPLC for the separation of isomers and eluted glycans were automatically fractionated onto a MALDI plate by a spotter (Accuspot ; Shimadzu Corp.) . Then, a Mass spectrum corresponding to each fraction time was obtained automatically, and the intensity maps of the mass values as a set of candidates for next MS^n analysis were created. Finally, MS^n analysis was performed semi-automatically on all the found precursors and isomer structures were identified by AGA2.

We applied the workflow to human immunoglobulin and erythropoietin, and obtained successful results.

The result of these glycoproteins indicates that our practical workflow could be applicable to multiple purposes including an biomarker discovery, quality assurance study of antibody-drug, and other screening researches of glycosylation.

References :
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PTu-042
13:30 – 14:40

Negative-ion fragmentation of neutral N-glycans derivatized with 3-aminoquinoline and other non-acidic reagents

Takashi Nishikaze, Kaoru Kaneshiro, Shin-Ichirou Kawabata, Koichi Tanaka
Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation, Kyoto, Japan

Keywords:
MALDI, N-Glycan, On-target 3AQ derivatization, Negative-ion mode, Fragmentation

Novel aspects:
The negative-ion fragmentation behavior of deprotonated or anion adducted N-glycans derivatized with non-acidic reagents depends on the structure of the reducing-terminal GlcNAc residue (opened or closed) and the anions used.

Abstract:
<Introduction>
Tandem mass spectrometry combined with collision-induced dissociation (CID) has been widely used to obtain structural information of glycans. In general, positive-ion CID usually leads to cleavages of glycosidic bonds thereby providing information simply on sequence and composition of *N*-glycans. Negative-ion fragmentation of underivatized *N*-glycans, on the other hand, has been proven to be more informative as it defines structural features such as the specific composition of the two antennae, the location of fucose, and the presence or absence of the bisecting *N*-acetylglucosamine (GlcNAc) residue. For glycan analysis, derivatization via reductive amination is often employed, but little is known about the influence of derivatization on negative-ion fragmentation. In our previous study, we have developed an on-target derivatization method for highly sensitive detection of glycans using 3-aminoquinoline/alpha-cyano-4-hydroxycinnamic acid (3AQ/CHCA) liquid matrix [*Anal. Chem.* 83 (2011) 3663-3667]. In this study, we evaluated the suitability of on-target 3AQ-derivatized *N*-glycans for the structural characterization based on negative-ion fragmentation. In addition, the fragmentation behavior of 3AQ-derivatized *N*-glycans was compared with *N*-glycans derivatized with other non-acidic reagents.

<Methods>
For on-target 3AQ derivatization, underivatized *N*-glycan was mixed with 3AQ/CHCA liquid matrix on a MALDI target, and the target was then left for 60min at 60°C on a heating block. 2-Aminobenzamide (2AB) and 2-pyridilamine (2PA) derivatized *N*-glycans were mixed with G_3CA liquid matrix, which consists of *p*-coumaric acid and 1,1,3,3-tetramethylguanidine. Mass spectra were acquired using a MALDI-QIT-TOF-MS (AXIMA-Resonance, Shimadzu/Kratos, UK).

<Results and Discussion>
In this study, derivatization with acidic reagents such as 2-aminobenzoic acid (2AA) was omitted from consideration. It has been indicated that negative-ion CID spectra of 2AA derivatives lack many important "diagnostic" product ions due to the localization of the negative charge on the acidic group of the derivative. Firstly, we compared negative-ion low-energy CID spectra of underivatized, 2AB-, 2PA-, and on-target 3AQ-derivatized NA2 glycans using a MALDI ion trap instrument. Negative-ion MS^2 of on-target 3AQ-derivatized *N*-glycans exhibited simple and informative spectra similar to those of underivatized *N*-glycans. It showed product ions by cross ring cleavages of the chitobiose core and ions specific to two antennae, D and E ions. It was shown that an interpretation of diagnostic product ions suggested for underivatized *N*-glycans could be directly applicable to on-target 3AQ-derivatized *N*-glycans. On the other hand, conventional 2AB- and 2PA-derivatized *N*-glycans gave complicated spectra which consist of extensive signals formed by dehydrations and multiple cleavages. These unfavorable product ions could be eliminated by omitting the reduction process, which is involved in the conventional derivatization method via reductive amination, resulting in spectra similar to that obtained from underivatized *N*-glycans. The difference in fragmentation behavior seemed to come from the structural difference of the reducing-terminal GlcNAc ring (closed or opened), rather than the structure of the aglycone moiety of derivatized *N*-glycan. On-target 2AB or 2PA derivatization approach was investigated as an alternative for the on-target 3AQ derivatization. However, complete derivatization was difficult, and ion yields were significantly low. There was no advantage over the on-target 3AQ derivatization. The fragmentation behavior of anionic adducts of 3AQ-derivatized *N*-glycan was also investigated and it was found that phosphate adduct shows important diagnostic fragments of *N*-glycans with high sensitivity. In conclusion, on-target 3AQ derivatization using phosphate doped 3AQ/CHCA liquid matrix was found to be suitable for both sensitive detection and structural characterization of *N*-glycans based on negative-ion CID fragmentation.

PTu-043
11:10 – 12:20

Atomic metal anions: one-step dehydrogenation and defluoronation of linear and cyclic hydrocarbons, alcohols and their fluoronated analogues

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Keywords:
metal, anions, dehydrogenation, defluorination, ligand

Novel aspects:
This work is the first extensive examination of the reactions of atomic metal anions, and demonstrates new reactions with alcohols, alkanes and fluorinated systems.

Abstract:
The generation and reactivity of gas-phase metal cations has been a focus of mass spectrometry related studies for quite some time. This has mostly been related to adduct formation and their involvement as reagents in chemical ionization processes. Our research group has recently discovered a way to generate a wide variety of metal anions (Na^-, K^-, Rb^-, Cs^-, Fe^-, Ni^-, Co^-, Cu^-, Ag^-) using a simple commercial electrospray mass spectrometer. The metal anions were all generated by the decomposition of metal cation carboxylate complexes. Owing to the previous difficulties associated with generating these unique species, the idea of reactive gas phase atomic metal anions has been relatively untouched. We will first present the results of theoretical calculations on the precursor metal-oxalate anions with a view to understanding the formation of the metal anion upon CID of the complex. The performance of a wide selection of methods was assessed and a robust computational approach to these systems was established. Clear from the calculations is that the situation for the transition metal anions is complicated by several low-lying electronic states, making the computational estimate of their properties a multi-determinant problem. None-the-less, evidence is seen for the insertion of metal atoms into the C-C bond in the oxalate dianion, a reaction that leaves the metal already primarily negatively charged. In some cases, the calculated structure is essentially a MCO_2 anion interacting with a neutral CO_2 molecule at some distance. Experimentally, this study investigates the previously unexplored reactivity of atomic metal anions produced in the source of a triple quadrupole mass spectrometer with several volatile organic molecules. One class consisted of the reaction of Fe- with the primary alcohols methanol through heptanol, secondary alcohols 2-propanol and 2butanol and the tertiary alcohol t-butanol. Dehydrogenation of the alcohols (except for methanol) was demonstrated through the observation of FeH_2^- in the resulting mass spectrum. Labelling studies showed that this dehydrogenation takes place in the reaction of Fe^- with a single alcohol molecule, and not in the two-step process previously observed for metal carbonyl anions such as $Fe(CO)_2^-$. In the case of the smaller alcohols methanol, ethanol and propanol, $FeOR^-$ and $FeOH^-$ anions are observed. Fe^- was also observed to dehydrogenate alkanes in a similar manner. Theory has been applied to chart the mechanism of the dehydrogenation reaction and the results will be presented in conjunction with rate constant measurements on an ion trap mass spectrometer. Metal anions have also been allowed to react with fluorinated ring-systems such as per-fluorophenol and hexafluorobenzene. Here there is evidence for competitive deprotonation of the phenol OH group and F^+ abstraction from the ring.

PTu-044
13:30 – 14:40

Energetics of dissociation of polycyclic aromatic hydrocarbons in interstellar environments

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Keywords:
RRKM interstellar ISM PAH energetics

Novel aspects:
Using Tandem MS and iPEPICO to accurately determine key bond strengths related to the interaction of H and H 2 with small PAHs of interstellar interest

Abstract:
Recently, great interest has evolved in the chemistry of the interstellar medium (ISM) ; especially the unidentified IR bands (UIRs) . UIRs, observed in a variety of environments, are believed to derive from neutral and ionic large polycyclic aromatic hydrocarbons (PAH) and their fragments. If they are responsible for UIRs, then PAHs would account for up to 20% of the organic carbon found in the ISM.

We are investigating small PAHs (naphthalene (NAP) , anthracene (ANT) , phenanthrene (PHE) and pyrene (PYR)) as well as some dihydro- equivalents (1,2-dihydronapthalene (DHN) , 9,10-dihydroanthracene (DHA) and 9,10-dihydrophenanthrene (DHP)) using imaging photo-electron photo-ion coincidence spectroscopy (iPEPICO) and electron impact mass spectrometry (MS) ; both mass analyzed ion kinetic energy spectrometry (MIKES) and collision induced dissociation (CID) . Experiments were performed at different ionization energies to produce breakdown diagrams for the various fragments. These diagrams are then fit using RRKM theory to determine the zero Kelvin activation energy (E_0) and the entropy of activation (ΔS) ; these results are then compared and discussed.

To date, all iPEPICO and MIKES results have been collected and the branching ratios have been measured in order to construct their breakdown curves. Looking at the fitted values of E_0 for the molecules it can be seen that there are notable trends when looking at the PAHs, and when comparing them to the dihydro-PAHs.

H loss is the dominant fragment for all molecules studied. For PAHs E_0 values are 4.20 +/- 0.04 eV and 3.92 eV and $\Delta S$are 2 +/- 2 $JK^{-1}mol^{-1}$, -0.03 $JK^{-1}mol^{-1}$ for NAP and ANT respectively. This indicates that as PAHs increase in size, there is a decrease in the E_0. ΔS values close to zero shows that the fragments are formed through bond cleavage.

H loss from dihydro-PAHs is more complicated, with two fragmentation channels resulting in the same fragment. Both DHN and DHA show a change in entropy ; which is indicative of a different fragmentation pathway. For DHN, the two sets of values for H loss are ; E 0 values of 2.34 eV and 2.22 eV and ΔSvalues of - 6 $JK^{-1}mol^{-1}$and -16 $JK^{-1}mol^{-1}$ for low and high energy respectively. This change has also been observed in DHA, which is still being investigated.

Another dominant fragment for PAHs is ethyne loss. The E_0 and ΔS values for ; NAP are 4.12 +/- 0.05 eV and 2 +/- 2 $JK^{-1}mol^{-1}$, and for ANT are 3.82 eV and 4 $JK^{-1}mol^{-1}$. E_0 again shows the inverse relationship to increasing size while there is little difference between ΔS.

The organic fragment for the dihydro-PAHs is methyl loss. The E_0 and ΔS values for DHN are 2.00 eV and -36 $JK^{-1}mol^{-1}$, and for DHA are 1.72 eV and -30 $JK^{-1}mol^{-1}$. A large degree of rearrangement occurs during the dissociation ; this can be partially explained by the formation of the methyl fragment, but it is also possible that the resulting ion has a significantly different structure from the parent ion. This is still being investigated.

NAP also loses H_2, both concertedly and consecutively as 2 H. E_0 of the two pathways are ; 4.77 +/- 0.062 eV for concerted loss and 4.20 +/- 0.037 eV and 3.26 +/- 0.099 eV (giving E_{0total} = 7.46 +/- 0.11 eV) for consecutive loss. This would indicate that it is more favourable for both hydrogen atoms to be lost simultaneously, which would be a good indication of the loss of H_2. When ΔS is compared, 12 +/- 4 $JK^{-1}mol^{-1}$ for concerted versus -18 +/- 7 $JK^{-1}mol^{-1}$ for the loss of the second hydrogen of the consecutive loss. This also shows that the concerted loss is more favourable.

PTu-045 Gas Phase Acid-Base Chemistry of Cysteine and Serine Homologs

11:10 – 12:20

John C Poutsma, Corbin Muetterties, Vincent Yannello, Ashley Janiga

College of William and Mary, WIlliamsburg, VA, USA

Keywords:
kinetic method, amino acid

Novel aspects:
First experimental study of the gas phase acid base properties of homologs of cysteine and serine.

Abstract:
The gas-phase proton affinities and gas-phase acidities of extended homologs of cysteine (Cys) and serine (Ser) have been obtained using the extended kinetic method in an ESI-triple quadrupole instrument. Previous photoelectron spectroscopy and high-level theoretical calculations have indicated that the preferred site of deprotonation for cysteine is the side chain thiol rather than the carboxylic acid moiety. This arises from differential hydrogen bonding ability between the side chain S-H group and the carboxylic acid O-H group. We have been interested in the relationship between the structure of amino acids and their acid-base properties. To this end, we have been determining gas-phase thermochemical properties of non-protein amino acid analogs of the 20 common amino acids. Previous studies from our lab on a series of lysine homologs showed that the proton affinity is highly dependent on the length on the side chain due to difference in intramolecular hydrogen bonding capabilities. In contrast the acidities of these species do not show a clear trend with decreasing side chain length. They do however have very different intramlolecular hydrogen bonding interactions. We anticipated that lengthening the side chain in cysteine homologs would affect the relative stability of the different hydrogen bonding schemes.

We have measured the gas-phase acidity of two longer homologs, homocysteine (hCys) and 5-mercapto-norvaline (hhCys) using the extended kinetic method in an ESI triple-quadrupole instrument. In addition, we have carried out high-level hybrid density functional theory calculations (B3LYP/6-311++G**//B3LYP/6-31+G*) on the gas phase acidity of the these two homologs and find that a) the absolute acidity of hCys is the same (1397 kJ/mol) as that of cysteine (1396 kJ/mol), b) that the COOH group in hcys is slightly more acidic than the thiol group (1.5 kJ/mol), c) the acidity of hhCys is less than that of Cys and hCys (1408 kJ/mol) and d) the COOH moiety is the preferred site of deprotonation for hhCys. Proton affinities for these species have also been measured and are in excellent agreement with those calculated at theB3LYP/6-311++G**//B3LYP/6-31+G* level of theory. We find that the proton affinity increases from 903 kJ/mol for Cys to 934 kJ/mol for hCys to 943 kJ/mol for hhCys consistent with increasing intramolecular hydrogen bonding ability for the longer chain homologs. Similar experiments and theoretical calculations will be presented for homoserine (hSer) and 5-hydroxynorvaline (hhSer).

PTu-046 Mass spectrometric analysis of 1,3,5-Trinitroperhydro-1,3,5-triazine (RDX)

13:30 – 14:40

Sehwan Park[1], Soo Gyeong Cho[2], Eun Mee Goh[2], Sungman Lee[3], Sung-Suk Koh[3], Jeongkwon Kim[1]

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Keywords:
RDX, Ion trap, ESI-MS, Explosive

Novel aspects:
Mass spectrometric analysis of RDX using ESI-MS to investigate adduct ions of RDX, such as RDX + formate, RDX + acetate, and RDX + benzoate

Abstract:
1,3,5-Trinitroperhydro-1,3,5-triazine (RDX) is one of the most widely used explosives. In this study, RDX was directly infused into an electrospray ionization mass spectrometry (ESI-MS) system in negative ion mode. The RDX stock sample was prepared in a concentration of 1 mg/mL in acetonitrile (ACN) . Then, the RDX stock sample was diluted in ACN (100-fold dilution) prior to the analysis. Direct infusion of the diluted RDX sample provided an adduct ion of RDX + $HCOO^-$ where HCOOH is believed to be originated from the decomposition of RDX. In positive ion mode, no RDX signal was observed in the current direct infusion ESI-MS analysis. The RDX samples spiked with acetic acid or benzoic acid were also analyzed, which provide an adduct ion of RDX + $C_2H_4O_2^-$ (m/z 280.9) , RDX + $C_7H_6CO_2^-$ (m/z 343.1) . Analysis of RDX spiked with ammonium acetate also generated an adduct ion of RDX + $C_2H_4O_2^-$ (m/z 281.1) . The detailed experimental procedures and results will be provided during the presentation.

PTu-047 Formation of hydrated amino acid ions and estimation of their first hydrated shell

11:10 – 12:20

Mami Sakai, Kanako Sekimoto, Mitsuo Takayama

Yokohama City University, Yokohama, Japan

Keywords:

amino acid, first hydrated shell, collision-induced dissociation, ambient ionization

Novel aspects:

First hydrated shells for pos/neg phenylalanine ions were estimated by using the breakdown graphs obtained from CID spectra of hydrated phenylalanine ions.

Abstract:

Introduction

Many of the hydrogen bonds between water and individual amino acids lead to the native state of protein along the folding pathway. In recent years, investigating the hydrated structure of amino acids has become an interesting subject. High-pressure mass spectrometry is one of the ideal techniques for evaluating the enthalpy H for sequential hydration reaction and investigating the stability of hydrated amino acid ions. However, especially deprotonated amino acids have never been studied and only small water cluster can be measured by high-pressure mass spectrometry. Here we attempted to make an ambient ionization system for the formation of both positive and negative ions of phenylalanine and those hydrated clusters. Furthermore, the ambient ionization system coupled with a triple-quadrupole mass spectrometer was combined to collision-induced dissociation (CID) experiments to estimate the water molecule detachment energy of protonated and deprotonated phenylalanine, and for estimating its first hydrated shell.

Methods

Mass spectra were acquired on a triple-quadrupole mass spectrometer TSQ-7000 (Thermo-Fisher-Scientific, San Jose, CA). Ambient ion source was constructed from a needle (ca. 1 μm in the tip curvature radius) for micro-plasma ionization and a micro ceramic heater for rapid vaporization. The gap between the needle and the orifice plate of mass spectrometer was 3 mm, and the needle angle to the axis of orifice hole was varied in the range of $0-\pi/2$. DC voltages applied to the needle were -2.2kV and +3.4kV. The discharge was performed in the laboratory air with relative humidity of 28 %-65 % at 298 K and 760 torr. The orifice temperature was varied in the range of 333-373K. The pressure of argon used for CID was 1.5mTorr.

Results

The micro ceramic heater placed in the laboratory air became feasible to vaporize a powder of amino acids putted on the ceramic plate, i.e., $Jv > 0$ in the expression for MS ionization $Ji = I\,Jv$. The resulting amino acid vapor diffused into a micro-plasma region was positively and negatively ionized by ion/molecule reactions in ambient air, i.e., $I > 0$. The resulting atmospheric ions H_3O^+ and O_2^-, originated from ambient air, were used for ion/molecule reaction as reactant ions. Positive and negative ion mass spectra of phenylalanine (Phe) at the orifice temperature of 353K and a humidity of 60 % showed peaks corresponding to protonated and deprotonated molecules and their water cluster series $[Phe+H]^+(H_2O)_n$ (n= 0 -18, m/z 166-490) and $[Phe-H]^-(H_2O)_n$ (n= 0 -21, m/z 164-542), respectively. The CID spectra of the individual precursor ions $[Phe\pm H]^\pm(H_2O)_m$ were obtained, and the breakdown graphs for precursor ions were drawn on the basis of the CID spectra. The detachment energy $E_{n,n-1}$ of one water molecule from hydrated cluster ions $[Phe\pm H]^\pm(H_2O)_n$ under the CID conditions were estimated from the breakdown graphs. In the case of the precursor ion $[Phe+H]^+(H_2O)_{15}$, the detachment energy $E_{n,n-1}$ (n = 1 - 8) could be estimated and the discontinuous gap in detachment energy between $E_{4,3}$ and $E_{5,4}$ was observed. The result obtained suggested that $[Phe+H]^+(H_2O)_4$ was more stable than larger cluster size above n = 5. Furthermore, the average values of $E_{n,n-1}$ obtained by five precursor ions $[Phe+H]^+(H_2O)_{13\text{-}17}$ was linearly correlated with that in reliable $H_{n-1,n}$ values obtained with a high-pressure mass spectrometry. In the case of the precursor ion $[Phe-H]^-(H_2O)_{14}$, the detachment energy $E_{n,n-1}$ (n = 1 - 7) could be estimated and the discontinuous gap in detachment energy between $E_{3,2}$ and $E_{4,3}$ was observed. This result suggested that $[Phe+H]^+(H_2O)_3$ was more stable than large cluster size above n = 4.

PTu-048
13:30 – 14:40

Mass Spectrometry Studies of Metal Triflates and Triflimides and Their Interactions with Lewis Bases

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Keywords:
Triflates ; Triflimides ; Catalysts ; Lewis acids ; ESI-MS.

Novel aspects:
Mass spectrometric characterization by ESI-MS of metal triflates and triflimides ; Ligand competition method ; Quantitative parameter for the interaction between ligand and Lewis acids.

Abstract:
Metal trifluoromethanesulfonates (triflates M $(CF_3SO_3)_n$) and bis (trifluoromethanesulfonyl) imide salts (triflimides M $[(CF_3SO_2)_2N]_n$) are regarded as Lewis "superacids ". This feature is attributed to the specific electronic structure of the anions : a highly delocalized negative charge, and a resulting low coordination power, leaving a highly reactive cation. Such salts have remarkable catalytic properties.[1]
Exploration of new preparation routes of these salts is an active field, and methods for their thorough characterization are desirable. Under specific conditions (nitromethane as solvent, addition of strong neutral ligands) , electrospray ionization mass spectrometry (ESI-MS) and collision induced dissociation (CID) can afford useful analytical data on these salts.[2] In this method, the formation of positive ions is based on the displacement of one anion in the salt by neutral Lewis bases, such as phosphoryl compounds or amides. For example, the bonding of two molecules of triphenylphosphine oxide to indium triflate produces a singly charged positive ion by expulsion of one anion from the salt :
In $(CF_3SO_3)_3 + 2\ Ph_3PO \dashrightarrow [In\ (CF_3SO_3)_2(Ph_3PO)_2]^+ + CF_3SO_3^-$
This kind of adduct ion is often the base peak in the ESI (+) mass spectrum of mixtures of the phosphine oxide and metal triflates in nitromethane. Similar ions were observed in the Fast Atom Bombardment mass spectra of rare earth triflates adducts with different strong Lewis bases.[3] From these observations, with the aim of ranking the ligand affinities for various metal centers, a competition method using mixtures of two different ligands was developed. The relative intensities of the ionic species containing the different combinations of ligands generated an order of ligand relative affinities for several metal salts.[4]
This semi-quantitative method was recently improved. Modeling the effect of the ligand concentrations on the signal intensities led us to propose a quantitative parameter describing the relative affinity for the metal center.[5]
Quantum chemical calculations using DFT are currently carried out, in an attempt to better understand the meaning of these affinities.
In this communication, these ESI-MS results and the current quantitative developments of the ligand competition method will be presented.
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PTu-049
11:10 – 12:20

Mass spectrometric approaches to enantioselective complexation system in solution

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Keywords:
Chirality, Complexation, Isotope-Labeling, Enantiomer

Novel aspects:
Effects of ionization method such as FAB, ESI and MALDI on enantioselective complexation equilibrium system were clarified using the deuterium-labeled enantiomer method, comparing with that in solution evaluated by NMR.

Abstract:
Enantioselective complexation equilibrium system in solution, in which a chiral crown ether (enantiopure) binds to an enantiomers of chiral ammonium ions, was examined using FAB, ESI and liquid-MALDI mass spectrometry coupling with the enantiomer labeled guest method, referring to the results of NMR analysis of the solution state. The chiral crown ether (enantiopure, H) binds to ammonium ion of isopropyl (*R*) -D-phenylalanate (G_R^+) at K_R = 2 M^{-1} and (*S*) -L-phenylalanate (G_S^+) at K_S = 1 M^{-1} in dichloromethane-d_2/chroloform-*d* (v/v = 1 / 2) at 298 K, respectively. The concentration ratio of the resulting complexes changes theoretically depending on the association constants (K_R and K_S) , the initial concentration of H, G_R^+ and G_S^+.
In order to observe the corresponding complex ions in mass spectrometry, three component solution including an equimolar mixture of deuterium-labeled (d_7) G_S^+ and unlabeled G_R^+, and H was used, and the resulting diastereomeric complex ions, $(H + G_R)^+$ and $(H + G_{S\text{-}d7})^+$, were monitored in mass spectrometry and the relative peak intensity of the complex ions was compared with the concentration ratio in solution.
The chiral crown ether was synthesized as reported before. Deuterium-labeled (*S*) -phenylalanine isopropyl-d_7 ester hydrochloride was prepared by esterification of amino acid with deuterated isopropyl alcohol (d_7) . Unlabeled (*R*) -phenylalanine isopropyl ester hydrochloride was synthesized using isopropyl alcohol.
Sample solutions were prepared by mixing H solution and equimolar solution of $G_R^+Cl^-/G_{S\text{-}d7}^+Cl^-$. $[G_R^+]_0 = [G_{S\text{-}d7}^+]_0$ were the constant concentration conditions and only $[H]_0$ was changed in each ionization mass spectrometric experiment.
In FAB mass spectrometry (matrix : 3-nitrobenzyl alcohol) , the relative peak intensity of the complex ions, $(H + G_R)^+$ and $(H + G_{S\text{-}d7})^+$, changed in the same manner of the concentration ratio of the complexes in solution. On the other hand, in ESI (methanol/chloroform) and liquid-MALDI (tetra (*n*-butyl) ammonium salts) mass spectrometry, the changes of the relative peak intensity of the complex ions against the initial concentrations of H, G_R^+ and $G_{S\text{-}d7}^+$ were not in agreement with those of the concentration ratio of the complexes in solution.

PTu-050 Characteristic hydration of saccharides isolated from liquid beams

13:30 – 14:40

Namiki Toyama

Genesis Research Institute, Inc., Chiba, Japan

Keywords:
saccharide, hydration, hydrogen bond, liquid beam, IR-laser ionization

Novel aspects:
Hydration structure of conformational isomers of saccharides was distinguished by distribution of the number of water molecules in a cluster ion produced from an aqueous solution of the saccharide.

Abstract:
Functions and properties of saccharides have been widely studied because of their importance in biology, chemistry and pharmacology. The nature of the saccharides is influenced by interaction with surrounding water molecules, which form a hydrogen-bond network not only in a bulk solution but also in a binding site of a sugar binding protein in vivo. Inter- and intra-molecular hydrogen-bond interactions of the saccharides in aqueous solutions have been investigated by means of spectroscopic and thermodynamic measurements. The complexity of the hydrogen-bond structure is enhanced by structural variation and fluctuation of the saccharide molecules in the solution.

Microscopic information on the hydrogen-bond interaction in hydrated molecules has been obtained by an isolation of clusters and molecules from solutions into vacuum. A soft ionization method has been developed by utilizing a lamellar liquid flow in the vacuum (liquid beam) in combination with an IR laser ablation technique [1-3]. We have studied isolation of various neutral molecules from a liquid beam of aqueous solutions and proposed a mechanism for ejection of molecular species from the solutions underirradiation of an IR laser resonant to an OH stretching vibration of liquid water [4,5]. We have reported aggregation of arginine zwitterions and their hydrophobic hydration structure [4] and proton transfer between weakly hydrogen-bonding water molecules in liquid water induced by an IR-multiphoton excitation [5].

Here, I report about formation and characterization of hydrogen bonded cluster ions produced from a liquid beam of an aqueous solution of saccharides (*M*) under irradiation of an IR laser on the liquid beam. Hydrated D-glucose cluster ions, $H_3O^+M_m(H_2O)_n$ and $OH^-M_m(H_2O)_n$, were observed with the hydrated oxonium and hydroxide ions, $H_3O^+(H_2O)_n$ and $OH^-(H_2O)_n$, in time-of-flight mass spectra for the sample solution of 1-100 mM D-glucose. Ion intensity of the cluster ions was analyzed as a function of concentration of D-glucose in the source solution. Increase in the ratio of the D-glucose cluster ions was found with the concentration of D-glucose, whereas the ratio of $H_3O^+(H_2O)_n$ and $OH^-(H_2O)_n$ decreased and the total intensity of the ions was constant. On the basis of the result, I propose a reaction between glucose molecules and a ion-pair, H_3O^+---OH^-, produced by an OH-vibrational excitation of a water molecule in an aqueous solution of D-glucose. The product ions, H_3O^+M and OH^-M, are ejected from the liquid beam with accompanied neutral molecules surrounding the ion core.

This ionization method was applied to investigate effects of the OH group conformation on hydration of monosaccharides. The number of water molecules (*n*) in the cluster ion has been compared for the conformational isomers of D-glucose, D-mannose and D-galactose, which are different at conformation of OH (2) and OH (4) groups from that of D-glucose, respectively. The distribution of *n* is distinguished in the galactose cluster ions from other monosaccharides for both of oxonium and hydroxide ion adducts. The difference of the *n* distribution in the cluster ions is interpreted as a difference in anisotropy of hydration for the monosaccharides.

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PTu-051
11:10 – 12:20

Evaluation of intermolecular association of glycosaminoglycan oligosaccharides using nanoelectrospray ionization mass spectrometry

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Keywords:
Glycosaminoglycan, Oligosaccharide, Non-covalent complex

Novel aspects:
Non-covalent intermolecular association between glycosaminoglycan oligosaccharides could be evaluated quantitatively using nanoelectrospray ionization MS. Structural requirement for efficient association could be discussed.

Abstract:
Considerable interests have recently been paid for mass spectrometric evaluation of non-covalent interactions between biomolecules. Several studies have successfully demonstrated the potential of MS to analyze non-covalent complexes generating in solution. An outstanding example is the use of cold-spray ionization (CSI) -MS, which is powerful to detect weakly-associated complex or cluster consisting of nucleic acids, amino acids, steroids, and so on.[1)]
The challenge of the present study is to observe glycan-glycan interactions quantitatively, especially focusing on glycosaminoglycans (GAGs) . GAG is widely distributed in vertebrate tissues and body fluids, and it is well known to play crucial roles in many biological phenomena such as regulation of growth factors or cytokines, cell-cell recognition, and infection, through intermolecular association.
According to the past literature, some specific interactions were suggested to occur between hyaluronan (HA) and chondroitin sulfate (CS) , both of which are representative GAGs[2)]. However, no evidence has been shown so far to confirm direct binding of HA with CS.
A nanoelectrospray ionization MS was employed to analyze associations between HA tetrasaccharide and other structural variations of mono- and oligosaccharides. Each combination of saccharide samples was dissolved in water and diluted with methanol at 50 % before MS analysis. MS experiments were performed on LCMS-IT-TOF system (Shimadzu) with negative ion mode. Interaction strength of each saccharide combination was estimated by the required concentration of formamide added into the sample solution to dissociate the non-covalent complex. A successful result of this study was observation of the complex of HA and CS tetrasaccharides, suggesting HA can interact with CS directly in solution. It was also revealed that the affinity of each saccharide to HA tetrasaccharide was different depending on the saccharide length, or substitution of hydroxyl group. Based on these data, the present method was considered applicable to evaluate non-covalent interaction between GAG oligosaccharides. Our study will also provide useful information to propose a novel saccharide structure to achieve much potent association.

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PTu-052

13:30 – 14:40

Enantioselective binding characteristics of amino acid to chiral copper(II) complex using electrospray ionization mass spectrometry coupling with the deuterium-labeled method

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Keywords:
Enantioselective coordination, Amino acid, Chiral ligand, Chiral-discriminating ability, Metal complex

Novel aspects:
We prepared chiral ligand/metal complexes to discriminate enantiomers of amino acids, and the chiral discrimination ability of the metal/chiral ligand complex toward amino acid evaluated by using ESIMS.

Abstract:
Mass spectrometry is one of the powerful and useful tools for clarifying the coordination of metal complexes in organometallic chemistry field. We have studied an enantioselective coordination of chiral compound to chiral organometallic complex using mass spectrometry. Recently, we found that amino acids enantiodifferentiatingly formed the three component complex with chiral ligand/metal complex and the chiral-discriminating ability toward amino acid was evaluated using electrospray ionization mass spectrometry (ESIMS) coupling with the deuterium-labeled enantiomer method.
In this research, we designed novel chiral tetradentate ligands, of which backbone were ethylenediamine *N,N'*-diacetic acid. Two types of the ligands derived from *S*-alanine in four steps. These differed in terms of different carbonyl terminal structure of *S*-alanine, (*S,S*) **L1** and (*S,S*) **L2**, ($CH_3XCOC^*HCH_3N$ (CH_3) $CH_2)_2$ [X = O, **L1** ; X = NH, **L2**] .
Copper (II) chloride was chosen as center metal on the basis of pre-examination using mass spectrometry. *R*-amino acids (*R*-AA) and deuterium-labeled *S*-amino acids (*S*-AA-d_n) (alanine, valine, leucine, methionine, phenylalanine) were purchased. *n* represents the number of deuterium atoms.
Sample solutions were prepared by mixing copper (II) chloride, ligand (**L**) , and equimolar mixture of *R*-AA/*S*-AA-d_n in methanol, and the concentration was adjusted at 100 mM. Mass spectra of the sample solutions were measured with a Finnigan LCQ^{DECA} (Thermo Quest) equipped with ESI source. The operating conditions were set as follows : spray voltage, 5 kV ; capillary temperature, 200 °C ; sheath gas flow rate, 30 arbitrary unit. Sample solutions were injected at a flow rate of 3 mL/min.
In all measured mass spectra, the complex ions, [Cu/ (**L**) / (*R*-AA)]$^+$ and [Cu/ (**L**) / (*S*-AA-d_n)]$^+$, were detected successfully.
The relative peak intensity of the complex ions, (I [Cu/ (**L**) / (*R*-AA)]$^+$/I [Cu/ (**L**) / (*S*-AA-d_n)]$^+$: I_R/I_S value) , enhanced as increasing the content of the amino acid componentin the prepared sample solution, and eventually reached a constant value under the condition of more than 4 equivalent amino acid mixture for [Cu/ (**L**)] . As the I_R/I_S value may reflect the concentration ratio of the complex ion in the solution, the I_R/I_S value would be reasonably regarded as an index of chiral discrimination ability of the [Cu/ (**L**)] toward the amino acid. When the mass spectra were measured for the given amino acids under the ratio ([*R*-AA/*S*-AA-d_n] / [Cu/ (**L**)] = 1 / 4) , the I_R/I_S value were larger than 1.00. Thus, [Cu/ (**L**)] preferred *R*-amino acids in forming the three component complex.
The stoichiometry and structure of complexes were clarified using UV-vis, CD, and NMR spectroscopy in detail. As a result, the chiral-discriminating of the complexes toward amino acids would be primarily occurred due to steric hindrance between the coordinating ligand and amino acid.

PTu-053
11:10 – 12:20

MECHANISTIC INVESTIGATION OF ECO-FRIENDLY IODINATION OF AROMATIC DERIVATIVES BY MASS SPECTROMETRY

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Keywords:
iodination, mechanism, phenol

Novel aspects:
Mechanistic elucidation of a well established aromatic iodination reaction.

Abstract:
Aromatic iodinated compounds have been used as valuable chemicals in pharmaceutical and agricultural chemistry and as building blocks in organic synthesis, especially for C-C and C-N bond forming reactions.[1,2] Some iodoarenes are also biologically active molecules used in medicine as drugs and diagnostic aids as radioactively labeled markers or contrasts.[3]
Among the approaches to synthesize halogenated aromatic compounds, the iodination promoted by iodide or iodine in combination with an oxidizer has stood out.[4,5] Following the growing concern over environmental pollution and sustainable development, "greener " iodination synthetic methods were recently developed, in particular aerobic oxidative procedures.[3] Although several protocols have been published regarding aqueous-medium for aromatic iodination, there is a lack of mechanistic evidences about this functionalization.
In this contribution we present the preliminary results of a mass spectrometry systematic mechanistic investigation of eco-friendly phenol iodination.
The protocol explored as study model was recently communicated as an efficient iodination method of phenols and other aromatic derivatives, employing iodine (I_2) and hydrogen peroxide (H_2O_2 30 %) in water.[6] Briefly, we treated phenol (1 mmol) with I_2 (2 mmol) and H_2O_2 30% (4 mmol) in water (5 mL) at 50 °C for 24 h (obtained 2,4,6-triiodophenol, yield about 80%) . To evidence the product ion of proposed iodine reactive species, blank reactions (without phenol) were also carried out in the same conditions (I_2/H_2O_2/Water and temperature) . Samples were collected at the beginning of the reaction and after 1, 3 and 18 h. Each sample was immediately analyzed by ESI-MS on a LC-MS8030 Triple-Quad mass spectrometer (Shimadzu) .The MS scan (m/z = 100 - 700) and product ion scan (m/z = 50 - 700) analyses were performed in positive and negative modes. The source parameters were screened to avoid any influence in detected species.
For the best of our knowledge all studies involving oxidative iodination protocols in aqueous medium have considered the iodonium ion (I^+) as the key intermediate for the electrophilic aromatic substitution. However, in our preliminary studies no I^+ related species was found by MS experiments. Additionally, we could detect several iodide containing structures (I^-, I_3^-, $[I\text{-}H_2O]^-$ and $[I\text{-}H_2O_2]^-$) . These findings are partially in agreement with some proposed reaction pathways described in the literature. Further investigations are been conducted in order to find other involved intermediates.

Acknowledgments : FUNDECT (MS) and SHIMADZU DO BRASIL.

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PTu-054
13:30 – 14:40

Comparing the dissociation energies of non-covalent complexes measured with BIRD and RRKM modelling of CID

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Keywords:
non-covalent complex, travelling wave ion mobility, CID, RRKM, BIRD

Novel aspects:
The effects of ion mobility upon dissociation energy measurements is explored in depth for a number of different and biologically relevant complexes

Abstract:
Several flavours of tandem MS dissociative methods are commonly used to investigate non-covalent complex stability in the gas phase, however the mechanisms can be quite different and therefore can give rise to different results. CID spectra at various collision energies were obtained for a number of non-covalent complexes on a Waters G2 Synapt mass spectrometer. Complexes included a short chain-variable fragment antibody with several related tri-saccharides, β-Lactoglobulin with fatty acids and a helical polysaccharides with fatty acids. A theoretical breakdown energy model based on RRKM theory was matched to experimental observations, yielding the 0K activation energy (E_0) and the entropy of activation. All systems studied have previously been analyzed using blackbody infrared radiative dissociation (BIRD) techniques, allowing for the relative stability rankings obtained using these 2 dissociative mechanisms to be compared.
The effects of ion mobility upon the internal energy distributions of the reacting non-covalent complexes is also explored, specifically in regards to the pressure of Helium, which is shown to have a drastic cooling effect upon the complexes.

PTu-055
11:10 – 12:20

Molecular recognition in gas phase: a method for quantitative analysis of protein-ligand interactions energy by CID-MS.

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Keywords:
Native mass spectrometry, energy transfer model, non-covalent interactions, protein-ligand interactions, molecular recognition

Novel aspects:
New model for calculation of gas-phase binding energy using CID mass spectrometry.

Abstract:
Molecular recognition is a process crucial for the majority of cellular functions. Better understanding of factors that play key roles in the interactions of biomolecules can provide means for broadening our knowledge about living organisms and developing new potent drugs. Two such major key-factors are the dynamics of the interacting molecules [1] and their interplay with aqueous environment [2]. The latter will be addressed here, both experimentally and theoretically. Vascular endothelial growth factor (VEGF), being an important therapeutical target related to the suppression of the tumor growth, was used as a model protein for this study.

Mass spectrometry provides a perfect opportunity to address the question about the role of the solvent in protein-ligand interactions because it allows studying molecules in the absence of their aqueous environment. For the present study, the library of 5 cyclicpeptidic ligands was prepared. Their affinities to VEGF in solution were determined with NMR chemical shift perturbation (CSP) technique. Then VEGF-ligand complexes were analyzed using the Electrospray ionizationquadrupole time-of flight (ESI-qTOF) mass spectrometer using collision induced dissociation (CID) in order to establish the ranking of the complexes according to their stability in gas-phase. It turned out that rankings of ligands in solution and gas phase are mutually opposite, i.e. the peptide with lowest solution affinity to VEGF forms the strongest complex in the gas phase, and vice versa. After calorimetric study of the system it was concluded that the enthalpic component of Gibbs energy of binding, that plays dominant role in stability of the noncovalent complex in gas phase, grows with decrease of affinity in solution, thus allowing the weakest ligand to form the strongest complex in gas phase [2].

To be able to compare directly the enthalpy binding and gas-phase binding energy a method is needed to calculate the latter from experimental data. Unfortunately, to-date there is no method to quantify gas-phase stabilities of noncovalent protein-ligand complexes from CID data. We propose a model that uses readily available properties of the interacting molecules, such as collision cross section or density of energy states, as input data, and allows modeling the "slow heating " of molecular complex during the CID [3]. The model takes into account that both excitation and relaxation of the system can take place upon collision with buffer gas molecule. The model can predict the amount of kinetic energy of the complex that is transferred to the internal energy under the certain conditions (buffer gas type, pressure, path length etc.) If the calculation was done under conditions, under which 50 percent of the complex are decomposed in the gas phase, the result of calculation can be taken as a gas phase stability of the complex. The model was verified with molecular dynamics simulations.

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PTu-056 Gas-phase basicity of 2-furaldehyde

13:30 – 14:40

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Keywords:

2-furaldehyde, extended kinetic method, proton affinity, gas-phase basicity, mass spectrometry

Novel aspects:

This study represents the first estimate of the gas-phase basicity of 2-furaldehyde, determined by the extended Cooks's kinetic method from ESI-TQ tandem mass spectrometric experiments along with theoretical calculations.

Abstract:

2-Furaldehyde (2-FA), also known as furfural or 2-furancarboxaldehyde, is an heterocyclic aldehyde that can be obtained from the thermal dehydration of pentose monosaccharides. This molecule can be considered as an important sustainable intermediate for the preparation of a great variety of chemicals, pharmaceuticals and furan-based polymers. Although the great importance of this molecule, its gas-phase basicity (GB) has never been measured. In this work the GB of 2-furaldehyde was determined by the extended Cooks's kinetic method from ESI-TQ tandem mass spectrometric experiments along with theoretical calculations. As expected, computational results identify the aldehydic oxygen atom of 2-FA as the preferred protonation site. The geometries of O-O-cis and O-O-trans 2-furaldehyde and of their six different protomers were calculated at the B3LYP/aug-TZV (d,p) level of theory ; PA values were also calculated at the G3 (MP2, CCSD (T)) level of theory. From the experimental proton affinity (PA) (847.9 ± 5 kJ mol^{-1}) and protonation entropy ($\Delta_p S$) (115.09 ± 10 J mol^{-1}) of 2-FA a GB value of 813.6 ± 2 kJ mol^{-1} at 298 K and a ΔH°_f value of533.0 ± 6 kJ mol^{-1} for protonated 2-furaldehyde were derived.

PTu-057
11:10 – 12:20

Non-covalent interaction between peptides and its effect on the dissociation process of Bradykinin Molecule

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Keywords:

Non-covalent Interaction ; Peptides ; Conformation ; Collision-induced dissociation mechanism ; Mass spectrometry.

Novel aspects:

The non-covalent interaction between peptides and the conformation mechanism of Bradykinin, and also the effect of non-covalent interaction on the collision-induced dissociation process of protein molecules.

Abstract:

The study of the gas-phase property of non-covalent complexes by the mass spectrometer is very important since it allows the exploration of non-covalent interactions in the absence of solvation effects. It permits a better understanding of the protein conformation and recognition mechanism[1-5].

The structure and conformation of proteins determine their biological functions. The interaction between peptides within a protein molecule is one of the major factors that influence its structure and conformation. In this work, Bradykinin (RPPGFSPFR) was chosen as a model to investigate the non-covalent interaction between peptides within the amino acid chain.

For investigating the possible non-covalent interaction between peptides of a Bradykinin molecule, several peptides which have the same amino acid sequence were synthesized. In the first fracture mode, we synthesized two peptides RS-6 (RPPGFS) and PR-3 (PFR) according to our requirement ; In the second fracture mode, two peptides RF-5 (RPPGF) and SR-4 (SPFR) were obtained. By removing the arginine at N-terminal or C-terminal, we designed and synthesized another four kinds of polypeptide, including PS-5 (PPGFS) , PF-2 (PF) , PF-4 (PPGF) and SF-3 (SPF) . In our experiments, any two kinds of these eight polypeptideswere mixed in a molar ratio of 1 : 1, respectively, in 90/10 methanol/water solution and then reacted at room temperature for 12 hours.

The evaluation of the non-covalent interaction by mass spectrometric experiments was performed using Thermo Finnigan LTQ ion trap mass spectrometer equipped with an electrospray ionization source. In the experiments, the electrospray capillary voltage was set to 3.5kV and the capillary temperature was maintained at 350&#8451 ; . The sample was introduced via a syringe pump at a flow rate of 3 μL/min.

Electrospray ionization mass spectrometry (ESI-MS) results revealed that the two peptides obtained in each mode can form non-covalent complex. In the first fracture mode, RS-6 and PR-3, PS-5 and PR-3 can form 1 : 1 complexes, while there is not any non-covalent binding between peptides RS-6 and PF-2,PS-5 and FP-2. And in the second fracture mode, there is always 1 : 1 non-covalent binding formed between two peptides, RF-5 and SR-4, PF-4 and SR-4,RF-5 and SF-3,PF-4 and SF-3, no matter whether the N-terminal or C-terminal arginine was removed.

The non-covalent binding between two peptides was further confirmed by collision-induced dissociation (CID) in a tandem mass spectrometer. It is demonstrated that the binding strength of RS-6 + PR-3 and RF-5 + SR-4 complexes is much stronger than other peptide complexes. These results are in accordance with the conformation of Bradykinin.

This study provides important and reliable information for the non-covalent interaction between peptides and the conformation mechanism of Bradykinin, and also the effect of non-covalent interaction on the collision-induced dissociation process of protein molecules.

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PTu-058
13:30 – 14:40

A continuous flow system for quantitatively preparation and isotopic measurement of radiogenic argon.

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Keywords:
isotope ratio mass spectrometer

Novel aspects:
new method for measurement of 36Ar and 40Ar isotopes for potassium argon (K/Ar) geochronology

Abstract:
Isotope composition of noble gas is widely used for geochemical and geochronological studies. Argon isotope ratios are measured with specialized mass spectrometers. Some new models of this technique have been produced in recent years. Having high-technology achievements for measurement isotope ratios of ultra-small quantity noble gas, these technique keeps significant requirement for vacuum system to achieve extreme high vacuum and measurements of isotope ratios in static mode. Maintenance of running conditions is a challenging task. Preparation systems for liberate argon from samples with the extremely low blank are not simple.

Here we present a new method for measurement of ^{36}Ar and ^{40}Ar isotopes for potassium argon (K/Ar) geochronology that can be realize with conventional isotope ratio mass spectrometer operating in a dynamic mode. We also present a laser preparation system where the extraction and cleaning argon is performed in a continuous flow (CF) of helium. The preparation system is attached to a mass spectrometer (MS) via open-split mode. The CF-MS method is an alternative to measurement of argon isotopes in a static mode and can be used for the analysis of radiogenic argon of $nx10^{-12}$ g with the same sensitivity and accuracy as a static mode. The method of argon isotope measurement in a continuous flow is simpler and more reliable compared to a measurement method operating in a static mode.

CF-MS system includes a mass spectrometer MAT-253 (Thermo Fisher Scientific) equipped with Faraday detectors at m/z 36, 37, 38, 39 and 40 connected to high-gain electrometers of $1 x10^{11}$ Ohms for ^{40}Ar and $1 x10^{12}$ Ohms for all other. The sample and reference argon enters into the ion source of the MS with a helium flow through fused silica capillary. The reference is a mixture of air argon and helium in the ratio of about 1 : 100 stored in gas-cylinder under pressure ~ 80 atm. It is connected to a dosing system to produce equal doses of the reference. A single reference dose contains 0.652 ± 0.001 ng of ^{40}Ar. The reference is used to calculate a quantity of radiogenic argon liberated from samples. This is provided by comparing the peak areas of the reference and sample, which are following each other in order of reference sample reference with a minimum time interval within a single chromatogram. This method, using of air argon as the reference gas has an advantage over conventional method of 38-Ar isotopic dilution : 1) there is no contamination of the sample by $^{40}Ar/^{36}Ar$ incoming from a tracer 38-argon ; 2) aquantity of argon in a dose is invariable for several years 3) availability of $^{40}Ar/^{36}Ar$ air ratio monitoring by reference gas.

Pretreatments techniques. Rapid and quantitative liberating of argon from samples is provided by the CO_2 laser heating within a multiple-charge chamber. The sample chamber is equipped with a built-in heater to remove a sorbed gas and flushed with a stream of clean He ~50 mL/min. Laser liberating sample argon are transferred by He flow to a cryogenic purification system and then to a cryo-focusing trap where sample argon is collected by a molecular sieve at liquid nitrogen temperature. After defrosting of cryo-focusing trap the short-pulsed argon are transferred to a capillary column. The column separates the argon from contaminations and produces a shape of peak to integrate ion currents of masses 36 and 40 by multiple Faraday detectors. Due to the short-pulsed output of argon, the value of signal / instrumental noise relationship is increased if to compare its with a measurement in a static mode for same sample sizes. All-around analysis take up 10-15 min. The background signals were $5 x10^{-4}$ng for ^{40}A rand $6 x10^{-6}$ng for ^{36}Ar.

PTu-059
11:10 – 12:20

High precision analysis stable isotopes of sulfur (32S, 33S, 34S) using UV laser fluorination continuous He flow mass spectrometry

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Keywords:
mass spectrometry, stable isotope

Novel aspects:
New method high precision analysis stable isotopes of sulfur (32S, 33S, 34S) using UV laser fluorination irm-GC-MS

Abstract:
During the last decades, significant advances in the field of stable isotope geochemistry have resulted from the development and application of systems, which are based on in situ laser extraction procedures. Current laser techniques make it possible to analyze $^{34}S/^{32}S$ isotope ratio with good spatial resolution (Crowe et al., 1990 ; Kelley and Fallick, 1990) . Nevertheless, systematic experimental work has shown that the most commonly applied laser extraction technique results in a systematic fractionation of the $d^{34}S$ values. Another recent advance is the development of continuous He flow techniques that use a UV laser ablation and gas chromatograph for online purification of gases, which allows to achieve precise analysis stable sulfur isotopes at nanomole level samples (Ono et al., 2006) . The technique involves fluorination of sulfide (silver sulfide or pyrite) , and separation of product gas by gas chromatography. The basic sources of error with continuous helium flow is found to be tailing of the major ion beam ($^{32}SF^{5+}$) into beams ($^{33}SF^{5+}$) , which results in distortion of the measured $\delta^{33}S$.
We have developed a method for determination of $^{34}S/^{32}S$, $^{33}S/^{32}S$ isotope ratios in sulfur-bearing minerals using UV laser ablation. Our equipment consists of small volume stainless steel chamber, a nickel reactor and six ports Valco valve with a trap for cryogenic separation SF_6. Helium (flow 40 cm^3/min) passes through the chamber and nickel reactor. A photoablation of the sample surface formed an aerosol which in a continuous He flow passes through the reactor with ~ 2 % admixture of BrF_5. Aerosol are fluoridated at 350 ^{0}C. Total SF_6 are condensed to a trap with liquid nitrogen. The trap is heated stepwise and cryogenically purified SF_6 are entered through chromatographic capillary column in the ion source. Purification of microquantity of SF_6 from other gases was carry out by cryogenic separation and chromatographic capillary column. Between the ion source is placed a special interface. The interface allows you to enter SF_6 after pumping helium and avoid distortion of the measurement results $\delta^{33}S$. Measuring the ratios of sulfur isotopes are carried out on a MAT-253 mass spectrometer.
Samples for in situ analysis were prepared as polished, 1 - to 2 -mm thick small slabs, up to 1.5x1.5 cm. in largest dimension. The slabs were placed in the sample. Reference SF_6 gas, with known isotopic composition, was used for standardization. Additionally, the chamber is loaded with standards IAEA-S1, IAEA-S2, and IAEA-S3. For making spot analysis of individual standards IAEA, samples of IAEA were placed into stainless steel (alloy SS316) cups with 2 mm hole in diameter. NewWave UP213 Nd : YAG laser (4 ns pulse length, wave-length 213 nm) was used as the ablation source for analysis of solid samples. The repetition rate of the laser we generally used 10 Hz. Ablation spot diameter ranges was between 50 and 200 mm. In our system to obtain the amplitude of 1 Volt signal (for pyrite) 150 laser pulses is sufficient at a resolution of 100 mm and range of fluences 46 J/cm^2. For all other cases 300 pulses are required at the same power. Sulfur isotope data are presented using standard notation $\delta^{34}S$, $\delta^{33}S$ and $\Delta^{33}S$ where $\Delta^{33}S = [\ln(1+\delta^{33}S/1000) - \lambda * \ln(1+\delta^{34}S/1000)] * 1000$. $\Delta^{33}S$ describe the deviation of a $\delta^{33}S$ from a reference mass-dependent fractionation line. Data are presented relative to V-CDT. We report results to perform high precision (±0.1 - 0.2‰, SD for $\delta^{34}S$, $\delta^{33}S$ and ±0.05‰ for $\Delta^{33}S$) in situ sulfur isotope measurements by LA in a suite of well-characterized, isotopically homogeneous natural sulfide minerals, pyrite, sphalerite, galena and standards IAEA-S1, IAEA-S2, IAEA-S3.

PTu-060
13:30 – 14:40

MC-ICPMS: enhanced ion yields for high precision measurement of sub-fg Pu and high mass resolution for S isotope measurements.

Jerome A Johemko[1], Charles B Douthitt[2], Johannes Schwieters[3], Claudia Bouman[3], Nicholas Lloyd[3]

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Keywords:
MC ICP-MS, sub fg Pu, Nd in seawater, S isotopes

Novel aspects:
New geometry cones and improved pumping for greater ionization efficiency. 10e12 ohm feedback resistors.

Abstract:
Significant improvements in analytical performance of the Thermo Fisher model Neptune-Plus high-resolution multiple-collector inductively coupled plasma mass spectrometer (HR-MC-ICPMS) include improvements in sensitivity and enhancements to the dynamic range.
Improvements to the pumping system and cone design lead to increased ion yields, in some cases up to 4 % (relative to nominal values around 0.1 %) . Significant increases in sensitivity have been observed for both wet plasmas (from solutions) and dry plasmas (from laser ablation of solids) . The enhancements to sensitivity, when combined with the many favorable characteristics of MC-ICPMS, open up a challenging class of analytical problems which require analysis of low concentrations in small samples ("*measuring nothing in not very much* ") ,as demonstrated by the accurate and precise quantitation and measurement of isotope ratios of sub-fg levels of Pu in 100-micro liter samples. Other recent examples will be shown, including high precision measurement of Nd in seawater at low (5 ppb) concentrations. The increase in sensitivity also leads to improvement in counting statistics, leading to higher precision and, in some cases, allows measurements to be moved from SEMs into Faraday cups, also resulting in higher precision. This will be illustrated by measurements of the variation of $^{235}U/^{238}U$ caused by mass dependent fractionation, a measurement which also exploits the large dynamic range of the Neptune-plus.

The high mass resolution capability of the Neptune-Plus HR-MC-ICPMS (to remove polyatomic interferences) combined with the enhanced sensitivity (to compensate for the reduction in sensitivity that accompanies increase in resolution) allow exploration of the measurement of S isotopes for elemental and isotopic analysis. Using high mass resolution and amplifiers with 10e12 ohm resistors for isotope dilution measurements, researchers report 50 times higher detection efficiency and 100 times better precision for sulfur and $\delta^{34}S$ than the single-collector high-resolution sector field type ICP MS (HR-ICP-SFMS) . Reduction of blanks by >1 order of magnitude relative to ID-TIMS suggests that ID-HR-MC-ICPMS is the best method for measurement of very low concentrations of S. Coupling GC with MC-ICPMS has allowed, for the first time, compound specific $\delta^{34}S$ isotope analysis (CSIA) of volatile organic molecules, and the technique is currently being extended for more general use in the measurement of S isotope systematics.

PTu-061
11:10 – 12:20

A COMPARATIVE STUDY OF CALCIUM AND STRONTIUM METABOLISM IN THE SHEEP MODEL USING CA-41 AND SR-84 ISOTOPIC TRACERS

Fransiska Dewi[1,2], Tim Schulze-Koenig[3], Annette Liesegang[4], Johanna Irrgeher[5], Claudia Weigt[6], Gisela Kuhn[6], Hans-Arno Synal[3], Thomas Prohaska[5], Thomas Walczyk[1,2,7]

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Keywords:
bone, calcium, strontium, stable isotope, isotope tracing

Novel aspects:
The use of strontium stable isotopes as surrogate markers of calcium, which is safer and less expensive compared to calcium radio- and stable-isotopes.

Abstract:
^{41}Ca, a quasi stable calcium (Ca) isotope, is a promising tracer for labeling human skeleton that can be detected at high sensitivity using accelerator mass spectrometry (AMS) . Once the skeleton is labeled, changes in urinary tracer excretion can be used to assess Ca bone balance and turnover. Stable strontium isotopes (^{84}Sr and ^{86}Sr) may serve as surrogate markers due to similarities in Ca and Sr metabolism. They are non-radioactive and more easily detected than ^{41}Ca. This study was conducted in an adult sheep to compare Ca and Sr in terms of urinary excretion and bone deposition.

A 2 year old sheep received a single intravenous dose of ^{41}Ca (50 nCi) and ^{86}Sr (40 mg) . Over a period of 180 days, spot urine samples were collected to follow clearance of both tracers. Samples were analyzed for ^{41}Ca by AMS at ETH Zurich and for ^{86}Sr and element content at NUS by thermal ionization mass spectrometry (TIMS) and atomic absorption spectrometry (AAS) , respectively. After sacrifice, relevant bones were obtained (tibia, radius, femur, metatarsus, metacarpus, vertebrae) . Bones were cut into slices and samples of about 2 x 2 x 2 mm taken to assess spatial differences in tracer deposition using the same techniques. To achieve even higher spatial resolution (with spot size as small as 10 µm) , similar pieces of bone that came from adjacent areas to those analyzed earlier were subjected to Ca and Sr elemental analysis and Sr isotopic analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) at BOKU.

Analysis by using TIMS and AAS showed that deposition and excretion of both elements were highly correlated but they did not equal each other. In the skeleton, we found that Ca was preferentially deposited in bone relative to Sr and that more of both tracers were incorporated in parts of the skeleton with higher remodeling rates (more active sites) . The observed linear correlation between Ca and Sr deposition indicated that no site-specific discrimination occurred. Higher incorporation of tracers in the more actively remodeled sites emerged from LA-ICP-MS analysis. As the sheep reached early adulthood when the tracers were given, we found a higher tracer deposition along both sides of, but not directly on, the growth plate in the metaphyseal area as well as between the inner and outer circles of the shaft.

With regards to tracer excretion, Sr is known to be less well absorbed in the gut than Ca and excreted preferentially over Ca in urine. These differences were also reflected in our findings when the Ca and Sr tracer excretion was plotted against time post-dose. Three-compartmental model (SAAM II) was used to describe the long-term kinetics of uptake, distribution and excretion of Ca based on the excretion data collected.

In this study we have demonstrated the ability to detect minute amounts of both tracers in the skeleton and urine over extended periods of time. Deposition of both tracers in the skeleton was also highly correlated with each other. Their excretion in the sheep model was found to follow closely the compartmental model that was established earlier for human Ca metabolism. These findings would speak for a possible use of Sr stable isotopes as surrogate markers of Ca in human studies.

PTu-062
13:30 – 14:40

Recent advance in MC-ICP-MS as a tool for the absolute determination of isotopic composition of heavy elements

Masaharu Tanimizu

Japan Agency for Marine-Earth Science and Technology

Keywords:
atomic weight, absolute isotopic composition, MC-ICP-MS, mass-dependent discrimination

Novel aspects:
summarize recent advance in MC-ICP-MS for atomic weight determination

Abstract:
The precision of isotope ratios is now easily accessible to the level of better than 0.01 % using TIMS and MC-ICP-MS. Especially, isotope ratios of various elements can be now determined by MC-ICP-MS through its high ionization efficiency of the ion source. The ion transmission of the MC-ICP-MS from sample uptake to the detection of ion current is also improved by the several orders of magnitude against requirements for natural geological and environmental samples. However, there are several data regarding mass-independent behavior of MC-ICP-MS (Nd and W) . This feature is fundamentally different from the mass-dependent ion transmission point of view which has been accepted for a long time. This is not so serious to the relative isotopic analyses against an isotopic reference, but important to determine absolute isotope ratios, which have been determined through the evaluation of mass-dependent discrimination factor (K factor) from gravimetrically prepared calibration solutions. This is also potentially affected to the element doping technique to correct the K factor using isotope ratios of a neighbor element to analyte.
In this presentation, I will introduce several recent advances in MC-ICP-MS to the determination of standard atomic weight and its uncertainty originated from natural isotopic variation, and its limitation and future direction will be suggested from a personal point of view.

PTu-063
11:10 – 12:20

MEASUREMENT OF IRON TRANSFER FROM DIET TO BRAIN IN THE RAT MODEL

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National University of Singapore, Singapore

Keywords:
Brain iron uptake ; Negative thermal ionization mass spectrometry ; Stable iron isotope techniques

Novel aspects:
This is to the best of our knowledge the first study investigating the link between dietary iron and brain iron uptake in an adult rat model using stable isotope techniques.

Abstract:
Neurodegenerative disorders are on the rise with Alzheimer's disease (AD) and Parkinson's disease (PD) being expected to reach epidemic proportions in the coming decades. Iron deposits in brain in AD and PD patients led to the suggestion that iron is possibly involved in the pathogenesis of neurodegenerative disorders. At the same time, economic welfare and improved production methods have led to a sharp increase in consumption of dietary or supplementary iron over the past 50 years in industrialized countries and more recently in the developing world. Many lines of evidence point to iron induced oxidative stress and free radical damage as mechanisms that may lead to neurodegeneration. It remains to be seen, however, if an excessive iron intake is a risk factor to healthy individuals and if high body iron is paralleled by high iron deposits in brain.

In this study we describe a novel methodological approach using stable iron isotopes to determine directly the fraction of iron in the diet that has been transferred to the brain and other organs over time in the adult rat model. We used for the first time ever a continuous feeding approach in which a constant amount of a stable iron isotope (^{57}Fe) was administered into the drinking water daily with the rat chow diet to seven 6-8 month old adult male Wistar rats over 4 months. Iron isotopic analysis of organ tissues was conducted using Negative Thermal Ionization Mass Spectrometry (NTI-MS) .

Isotopic enrichment for the brain is 7%. Inter-individual variations between rats were relatively small as indicated by the relatively small standard deviations obtained for the brain across animals (8 %RSD) . The most surprising finding in our study is the observation that iron uptake by brain is considerable in normal adult rats. Although only 0.000537 ± 0.000076% of dietary iron intake could be traced in brain on average, which may appear negligible, this amount constitutes ca. 9% of total brain iron with figures being of similar order of magnitude for the other tissues under investigation (11.5-36.3%) . The abovementioned ca. 9% of brain iron could be traced back to the diet over a period of four months, a time span that compares to 7-10 human years.

Our knowledge of brain iron homeostasis and uptake is still very limited. Attention has been paid in the past primarily to the growing brain and impact of iron deficiency on brain development with radioisotope techniques. To our knowledge, no studies have been published to date focusing on iron accumulation in the brain of the adult rat. This knowledge gap can now be closed with the methodological approach we are presenting here. Contrary to radioisotopes, stable isotopes do not decay. This does not only eliminate health hazards and practical limitations in conducting feeding trials. Tracers can be fed over years, if deemed necessary, and samples stored without any time limit. Furthermore, long term continuous feeding studies allow us to determine brain iron balance reliably as opposed to the conventional single dose iron tracer administration with short-term observational period.

PTu-064
13:30 – 14:40

Elemental mapping of Eu, Yb, Hf, Pb, Th and U for zircon using high resolution laser ablation-ICP-Mass spectrometry

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Keywords:
mass-spectrometry, laser ablation, imaging, zircon

Novel aspects:
Two-dimensional mapping for isotopes with high resolution LA-ICP-MS techniques would reveal the trace elemental distribution mechanism during zircon formation in granites.

Abstract:
The laser ablation system attached to an ICP-mass spectrometry (LA-ICP-MS) has been widely used for elemental and isotopic analyses for solid and liquid samples. The laser ablation system utilizing a shorter wavelength and pulse width can minimize an elemental fractionation during both laser ablation and ionization processes, and improve transport efficiency of sample from the ablation area to the ICP ion source. Moreover, magnetic sector type ICP-MS instruments can provide both higher sensitivity and lower background counts, resulting in high spatial resolution of the LA-ICP-MS analyses. The high resolution ICP-MS would enable to conduct elemental and isotopic analyses and imaging with a laser pit size less than 10 μm, which may be comparable to an ion-beam size of secondary ion mass spectrometry (SIMS). In this study, we demonstrate that two-dimensional images for isotopes of ^{153}Eu,^{172}Yb, ^{178}Hf, ^{204}Pb, ^{206}Pb, ^{207}Pb,^{208}Pb, ^{232}Th, and ^{238}U, and ^{206}Pb/^{238}U ratio were yielded from the OD-3 zircon crystal with the LA-ICP-MS using a 2 μm laser spot size, and discuss the elemental distribution within zircon comparing the cathodo luminescence (CL) images.

The OD-3 zircon was collected from Mihara body of Kawamoto granodiorite distributed in Shimane prefecture, Japan. The zircons are euhedral to subhedral crystals with reddish color. The age of the Kawamoto granodiorite was dated to be 33 Ma based on the systematic comparison of zircon fission track and (SHRIMP, LA-ICP-MS or TIMS) U-Pb dating. The imaging was carried out using a Nu AttoM magnetic sector field-single collector-ICP-MS (Nu instruments, Wrexham, UK) equipped with a NWR193 laser ablation system (New Wave Research, Fremont, CA, USA), which utilizes 193 nm ArF excimer laser. The result shows that ^{153}Eu, ^{172}Yb,^{178}Hf, ^{206}Pb and ^{238}U were heterogeneously distributed within the zircon. The signal intensity of ^{232}Th shows non-linear correlation with that of ^{238}U, which was negatively correlated with the CL luminosity. Moreover, distribution of ^{178}Hf was clearly different from both the distribution of ^{238}U and CL images. The non-linear correlation could be explained either by changes in the ratio of the distribution coefficients (D_U/D_{Th}), or by diffusion properties of these elements during the crystal growth of zircon.

PTu-065
11:10 – 12:20

The estimation of the effect of initial 230Th disequilibrium on U-Pb dating of young zircon crystal by LA-ICPMS

Shu-hei Sakata[1], Hideki Iwano[2], Kenshi Maki[1], Takaomi D Yokoyama[1], Takahumi Hirata[1]

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Keywords:
zircon chronology, LA-ICPMS U-Pb age, 230Th disequilibrium, high-sensitivity measurement

Novel aspects:
Developments of high-sensitivity LA-ICPMS technique revealed the contribution of disequilibrium on 230Th onto the resulting U-Pb ages for the young zircons.

Abstract:
Recent developments in the laser ablation-ICP-MS technique make it possible to apply U-Pb dating method to young zircon crystals which are younger than 1 Ma. For the dating of young zircons, however, we have to consider a possible systematic error which can be originated from the effect of initial loss or enhancement of ^{230}Th in both the zircon crystal and source magma. In this case, the true ^{238}U-^{206}Pb age (T) can be obtained through the following equiation :
$^{206}Pb/^{238}U = [(\exp(\lambda_{238}T) - 1) + (\lambda_{238}/\lambda_{230})(f - 1)(1 - \exp(-\lambda_{230}T))\exp(\lambda_{238}T)]$,
where λ_{230} and λ_{238} are decay constant of ^{230}Th and ^{238}U, respectively, " f " represents the level of loss or enhancement of ^{230}Th, which is defined by the degree of Th-U fractionation between zircon and source magma $[(Th/U)_{zircon}/(Th/U)_{magma}]$. In this study, the $^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$ and $^{208}Pb/^{232}Th$ ratios for several young zircon crystals whose ages varies from 0.5 to 1 Ma, were measured by means of a high-sensitivity ICP-sector field-mass spectrometer coupled with laser ablation sample introduction technique. Based on the measured ratios, we tried to estimate the contribution of disequilibrium on ^{230}Th onto the resulting U-Pb ages for the young zircons through two different combination of the U-Pb and Th-Pb ages. First one is the conventional concordia-plot, and the second is the pseudo-concordia diagram ; plot of $^{207}Pb/^{235}U$ ratio against $^{208}Pb/^{232}Th$ ratio. If there is the effect of ^{230}Th disequilibrium, resulting data would leave from the concordia-curve and be plotted on the concordia-curve on the $^{207}Pb/^{235}U$ vs $^{208}Pb/^{232}Th$ diagram because the contribution of disequilibrium nuclide should be smaller for the ^{235}U-^{207}Pb and ^{232}Th-^{208}Pb decay series. Further details about the result will be discussed in our presentation.

PTu-066
13:30 – 14:40

Developing a human cancer diagnostics system based on the probe electrospray ionization-mass spectrometry and Bayesian statistics

Kentaro Yoshimura[1], Lee Chuin Chen[2], Mridul Kanti Mandal[3], Kunio Tanabe[1,5], Michio Hara[4], Hideki Fujii[4], Masayuki Takeda[6], Kenzo Hiraoka[3], Sen Takeda[1]

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Keywords:
PESI, Diagnostics, Cancer, Electrospray, Ambient

Novel aspects:
We established a rapid human cancer diagnostics system based on ambient ionization/mass spectrometry and statistics. Our system is applicable to raw specimens resected from patients without any pretreatments.

Abstract:
Probe electrospray ionization (PESI) is an ambient ionization technique that uses a fine stainless needle for pickup and ionization of samples [Hiraoka et al., 2007] . The needle has a diameter of 140 μm and its tip radius is sub-micrometer, and moves up and down along a vertical axis by a linear actuator to capture the sample from surface of specimen and generate the electrospray [Yoshimura et al., 2009] . The strength of PESI-mass spectrometry (PESI-MS) lies in low invasiveness, rapidity and no need for particular pretreatments, especially when applied to biological samples. In the present study, we constructed a prototype of cancer-diagnostic system using quadrupole mass spectrometer (LCMS-2020, Shimadzu) . The liquid chromatograph unit is disassembled and replaced with the PESI unit.

Previously, we reported real-time analyses of living mice that delineate the differences in lipid composition of hepatocytes between normal and steatotic mice [Yoshimura et al., 2011] . As a next step, we analyzed various human tissues to test its potential application to medical diagnosis. When we analyze the surgically extirpated human kidney, liver, pancreas, gall bladder, lymph node, and mucosal epithelium, unique mass spectral patterns were obtained from each specimens. These spectra were mainly derived from lipid components. Next, to ascertain whether or not cancer specific mass spectra exist, human clear cell renal cell carcinoma (ccRCC) was used as a positive control because of its well-known feature of lipid storage in the cytoplasm. As expected, we could successfully obtain distinct profiles of lipids in cancerous region, in which TAG dominated. Subsequently, mass spectra of non-cancerous and cancerous regions from 8 cases were subjected to PCA. Principal component scores from both specimens were clearly separated, the results suggesting the usefulness of lipid profiles for cancer diagnosis [submitted] .

To further sophisticate our diagnosis system, we employed the dual penalized logistic regression machine (dPLRM) , which is a Bayesian inference-based learning machine and automatically give a probabilistic prediction by referring the training data set [Tanabe et al., 2001] . Acquired raw data of ion intensities from ccRCC are exported and stored on a database format. This database is then used as training data set for dPLRM-based diagnostic algorithm. We performed a single-blind evaluation for ccRCC, and predictions by dPLRM were completely consistent with pathohistological diagnosis.

This algorithm does not require the annotation of cancer specific molecules (cancer markers) for prediction of cancer probability, because it recognizes the relative variations in the whole mass spectral pattern. To demonstrate the potential of our system on cancer diagnosis, we measure the mass spectra of human hepatocellular carcinoma (HCC) , which does not show any prominent peaks as seen in the RCC. Surprisingly, our system showed coincidence of diagnosis with that by pathohistology. These results collectively demonstrate the usefulness of PESI-MS to the human cancer diagnosis [in preparation] .

PTu-067
11:10 – 12:20

Ultrasensitive Multiplexed Detection of miRNAs Using Gold Nanoparticles and LDI-TOF MS

Hyunook Kang, Seol-Hye Hong, Woon-Seok Yeo

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Keywords:
miRNA, LDI-TOF MS, nanoparticles, quantification

Novel aspects:
This poster presents a new method for miRNA detection with both multiplexing and quantification capabilities. In addition, the limit of detection of the method is well below picomolar (pM) .

Abstract:
In this poster, we describe a simple, fast, and sensitive method for the multiplexed detection of miRNAs without the need of PCR or a labeling by combining self-assembled monolayers (SAMs) on gold and laser desorption/ionization time of flight mass spectrometry (LDI-TOF MS) . In our strategy, the target miRNA is captured specifically by the complementary DNAs immobilized on gold nanoparticles (AuNPs) and gold chips. AuNPs carry a large number of small molecules, called Am-tag, as a reporter for the detection of the target miRNA. The presence of miRNA in a solution is verified by mass peaks corresponding to Am-tag using LDI-TOF MS. For the multiplexed detection, we designed and synthesized four Am-tag molecules containing 0, 4, 8, 12 isotopes so that they have same molecular properties but different molecular weights. By observing and analyzing mass signals of these Am-tags on AuNPs decorated with different probe DNAs, miRNAs in samples can be easily discriminated and the relative amounts of miRNAs can be quantified. As a model case, we tested four miRNA, mir-365, mir-142- 3 p, mir-1207- 3 p, and let- 7 c. We believe that this strategy will provide a biological important tool for accurate, sensitive, rapid, and low-cost multiplexed detection of miRNA.

PTu-068
13:30 – 14:40

Mapping longitudinal proteomic expression levels in brain tissue for an amyloid Alzheimer's disease mouse model using shotgun based mass spectrometry

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Keywords:
Alzheimers ; Transgenic mouse ; proteomics ; CPE

Novel aspects:
The proteomic expression levels in brain tissue of an Alzheimer's disease mouse model were longitudinally studied using optimized extraction conditions and label free quantification

Abstract:
Alzheimer's disease (AD) is the most common form of dementia amongst the elderly, accounting for 60% of all diagnosed cases. Typically, diagnosis of AD cannot be made until the disease has progressed to the point that dementia is present. The neuropathology of AD is characterized by the aggregation and deposition of misfolded peptides and proteins, in particular, aggregated β-amyloid peptide in the form of extracellular plaques and hyperphosphorlylation of the tau protein in the form of intracellular neurofibrillary tangles in specific brain regions. Another key feature in the bimolecular processes underlying AD pathobiology is alteration of the membrane interactions in the brain tissue. This is confirmed by the fact that protein interactions are altered as a result of abnormalities in the membrane protein (MP) interactions. In the same way, degradation products from MPs may serve as diagnostic markers of disease. The advances in proteomics technologies, especially in liquid chromatography-tandem mass spectrometry (LC-MS/MS) , provide opportunities for the identification of disease-specific proteins and biomarker discovery. Despite a great progress has been made in brain research, comprehensive analysis of brain MPs remains a challenge due to their hydrophobicity, heterogeneity and low abundance.

We have successfully optimized cloud point extraction (CPE) methodology for the simultaneous extraction and enrichment of both the MPs and hydrophilic proteins from brain tissue followed by "shotgun " based MS analysis. High preconcentration coefficients, good selectivity and possibility to combine with various methods of downstream analysis are attractive features promoting the use of non-ionic surfactants to extract hydrophobic proteins which can be potential molecular markers of the disease from biological samples.

The present study was designed to longitudinally characterize the proteomic profile in the AD disease amyloid mouse model (Tg2576 APPSWE) to find proteins linked to AD. Transgenic mouse models of AD have served as valuable tools for investigating pathogenic mechanisms related to Aβ accumulation. The Tg2576 model, which have been used in this study develops many neuropathological features of Alzheimer's, including amyloid plaques, dystrophic neuritis and inflammatory changes, and it is one of the most well characterized strains of transgenic animals over-expressing human APP.

Brain tissue from the Tg2576 APPSWE mouse model and litter mate controls at the age of 12, 15, 18 and 22 months were cloud point extracted and phase separated using the non-ionic surfactant Triton X-114. The extracted protein fractions were delipidated and precipitated with tri-n-butylphosphate/acetone /methanol (1 : 12 : 1) . The proteins were tryptically digested on 3 kDa spin filters. The detection of peptides and analysis of their expression levels were done with nanoLC-MS/MS using a 7 T hybrid LTQ FTMS instrument together with a label-free approach. This longitudinal study of the Tg2576 mouse model gave 480-720 unique proteins identified/animal. The utilized CPE method yields a phase separation of the proteins into either a hydrophilic or a hydrophobic phase, which can be processed and analyzed individually. Eight out of 13 suggested synaptic degradation markers were identified in all samples. Label free quantification and multivariate data analysis of the extracted samples showed a good age correlation within each studied age group. However, clear age related trends could be found for the AD model. These trends were most distinct in the hydrophobic (membrane) fractions showing a clear decrease of the number of proteins and expression levels with age. The obtained results show the potential of longitudinal monitoring of neurodegenerative processes in disease progression.

PTu-069 Serum metabolome analysis for early detection of colorectal cancer

11:10 – 12:20

Masaru Yoshida[1,2,3], Shin Nishiumi[1], Yoshihiro Izumi[1], Atsuki Matsubara[1], Takeshi Azuma[1]

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Keywords:
serum metabolomics, GC/MS, colorectal cancer

Novel aspects:
GC/MS-based serum metabolomics could be used as a novel method for colorectal cancer screening tests.

Abstract:
To improve the quality of life of colorectal cancer patients, it is important to establish new screening methods for early diagnosis of colorectal cancer. We performed serum metabolome analysis using gas-chromatography/mass-spectrometry (GC/MS). First, the accuracy of our GC/MS-based serum metabolomic analytical method was evaluated by calculating the RSD% values of serum levels of various metabolites. Second, the intra-day (morning, daytime, and night) and inter-day (among 3 days) variances of serum metabolite levels were examined. Then, serum metabolite levels were compared between colorectal cancer patients (N=60; N=12 for each stage from 0 to 4) and age- and sex-matched healthy volunteers (N=60) as a training set. The metabolites whose levels displayed significant changes were subjected to multiple logistic regression analysis with the stepwise variable selection method, and a colorectal cancer prediction model was established. The validity of the prediction model was confirmed using colorectal cancer patients (N=59) and healthy volunteers (N=63) as a validation set.

The prediction model was composed of the 4 metabolites selected, and its AUC, sensitivity, specificity, and accuracy were 0.9097, 85.0%, 85.0%, and 85.0%, respectively, according to the training set data. In contrast, the sensitivity, specificity, and accuracy of CEA were 35.0%, 96.7%, and 65.8%, respectively, and those of CA19-9 were 16.7%, 100%, and 58.3%, respectively. At the validation set, the sensitivity, specificity, and accuracy of the prediction model were 83.1%, 81.0%, and 82.0%, respectively, and these values were almost the same as those obtained with the training set. In addition, the model displayed high sensitivity for detecting stage 0-2 colorectal cancer (82.8%).

Our prediction model established via GC/MS-based serum metabolome analysis is valuable for early detection of colorectal cancer and has the potential to become a novel screening test for colorectal cancer.

PTu-070
13:30 – 14:40

Analysis of Drugs and Steroid Hormones in Human Saliva and Serum using Column-switching Ultra-fast LC/MS/MS with On-line Analyte Purification

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Keywords:
Saliva, Serum, Drug, Steroid-hormone, Column-swiching

Novel aspects:
We developed an convenient analytical method for drugs and steroid hormones in saliva, substituting for blood, using column-switching LC/MS/MS and showed the correlation in those concentrations in saliva and serum.

Abstract:
Introduction :
Saliva which has a similar composition to blood can be used as a substitute for blood in medical screening, such as therapeutic drug monitoring and biomedical examinations. It is generally believed that concentrations of components in saliva depend on concentrations of protein-unbound components in plasma, and examining the correlation in concentrations is important. We have therefore analyzed several oral drugs and steroid hormones in saliva and serum of test subjects dosing the drugs by column-switching LC/MS/MS using an ultra-fast LC system and compared concentrations of the drugs and hormones in saliva and serum.

Methods :
Saliva and serum were collected from healthy adult volunteer-subjects. Informed consent was obtained from every subject prior to the dosage and collections at Hitachi Yokohama Hospital. After dosing an oral-drug with 100-mL water, saliva and serum were collected from subjects at 15 and 3 times, respectively, within about 9 hours. The unstimulated saliva were accumulated in the oral cavity and spat into plastic sample tubes in several batches for 15 minutes.
The saliva and serum were kept frozen at -30 &#8451 ; and thawed at room temperature. To analyze the drugs, the saliva and the tenfold-dilution serum with water were added to an equal amount of an LC-mobile phase solution, and vortex mixed. Samples were centrifuged and supernatant fluids were used for analysis. A series of saliva-based calibrator solutions and a series of diluted serum-based calibrator solutions were treated in the same way. To analyze the steroid hormones, the saliva and the tenfold-dilution serum with water were added to LC-mobile phase solution containing an internal standard and other preparations were almost the same as those used in drug analysis.
A LachromUltra ultra-fast liquid chromatograph system (Hitachi Hi-Technologies, Tokyo, Japan) , a 3200 Q TRAP tandem mass spectrometer system equipped with an ESI ion source (AB SCIEX, Massachusetts, USA) , and a Model 3011 six-port switching valve unit (Shiseido, Tokyo, Japan) were used in this analysis. The pre-columns for protein removal were Capcell Pak MF-C 8 or MF-SCX cartridges (4.0 mm i. d., 10 mm long, Shiseido, Tokyo, Japan) , the intermediate trapping column was an OASIS HLB column (2.1 mm 1.d., 20 mm long, Waters, Massachusetts, USA) . The main analytical column was a LachromUltra C18 column (2.0 mm i.d., 20 mm long, Hitachi Hi-Technologies, Tokyo, Japan) . The sample was introduced into the pre-column with the mobile phase for pretreatment (aqueous solution containing 0.1 % formic acid, 10 mM ammonium formate, or 5 mM ammonium acetate) , after removing proteins the flow channel was switched by the valve then the sample injected into the trapping column. After trapping objective substances, the substances were flashed with the mobile phase for analysis (water/methanol (30 : 70, v/v) containing 0.1 % formic acid, 10 mM ammonium formate, or 5 mM ammonium acetate) by flow channel-switching, using backflash method, then introduced into the analytical column and separated. The separated substances were ionized and measured by MRM.

Results and discussion :
The LLOQ of Sodium valproate, Etizolam, and Ibuprofen were 10, 0.05, and 1 ng/mL, respectively, with a signal-to-noise ratio of 10 : 1. The coefficients of variation (CVs) of Sodium valproate, Etizolam, and Ibuprofen (saliva-based calibrator solutions) were 1.6, 3.9, and 4.1 %, respectively. The LLOQ of progesterone was 0.025 ng/mL. The CV of progesterone was 2.3 %.
Correlation coefficients of concentrations in saliva and serum for Sodium valproate, Etizolam, and Ibuprofen were 0.77, 0.72, and 0.74. The correlation coefficient of concentrations in saliva and serum for progesterone was 0.83.

Conclusions :
Concentrations of components in saliva and serum showed good correlation. This method for saliva-screening has merits for young patients, such as babies and children.

PTu-071
11:10 – 12:20

Acylglycine: A Potential Biomarker for the Clinical Diagnosis of Inborn Metabolic Disorders

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Keywords:
Acylglycines, Inborn errors of metabolism

Novel aspects:
We developed a LC-MS/MS method for acylglycines determination with isomers and set up the reference intervals in pediatric Chinese.

Abstract:
Urinary organic acids, plasma amino acids and acylcarnitine profile analyses are the main tools used to diagnose inborn errors of metabolisms (IEM) . However, without metabolic decompensation, these parameters are often not helpful. On the other hand, in cases of IEM, acylglycines are consistently raised even when patients appear to be in remission. This study aims to set-up a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method for the determination of urine acylglycines, complementary to organic acid and acylcarnitine profiles, for the diagnosis of IEM. In addition, local reference intervals for various acylglycines are established by using this method. Acylglycines were isolated by solid-phase extraction, derivatized with n-butanol, separated by HPLC, and detected by ESI-MS/MS. Acylglycines were quantified with deuterated internal standards. Mean recoveries of acylglycines ranged from 90.2 to 109.3%. Within- and between-run imprecisions for all acylglycines have CVs less than 10%. Linear regression coefficients were greater than 0.99. Reference intervals were established according to CLSI guidelines by analyzing 204 samples from apparently healthy individuals less than 18 years of age. Partitioning into age group reference intervals was not indicated, according to the Harris and Boyd approach. In this context, a single reference interval for each acylglycine could be used. This method of urine acylglycines analysis is a powerful diagnostic tool, complementary to urine organic acids and plasma acylcarnitine profiling, for detecting certain inborn errors of metabolism.

Reference :
Fong, B.M.W. ; Tam, S. ; Leung, K.S.Y. *Talanta,* **2012,** *88,* 193-200

PTu-072
13:30 – 14:40

Clinical application of rapid bacterial identification using mass spectrometry

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Keywords:
rapid bacterial identification, MALDI-TOF MS

Novel aspects:
Clinical application of rapid bacterial identification using mass spectrometry

Abstract:
Background : In a clinical diagnostic microbiology laboratory, the current method of identifying bacterial isolates is based mainly onphenotypic characteristics, such as growth patterns on different media,colony morphology, Gram stain, and biochemical reactions.Collectively, these techniques can identify mostbacterial isolates accurately, but they are costlyand time-consuming. We prospectivelyassessed the performance of MALDI-TOF MS in identifying bacterial strains that are routinely isolated from clinicalsamples (Anal Bioanal Chem 2011 ; Anal Bioanal Chem 2012) . We also examined positive blood culture bottles for which a rapid decision was required.
Methods : Bacterial colonies obtained from a total of 468 strains of 92 bacterial species isolated at the Department of Clinical Laboratory at Chiba University were directly placed on target MALDI plates, followed by addition of CHCA matrix solution. For identification of bacteria from positive blood culture bottles, samples of approximately 3 mL were recovered from positive blood culture bottles and injected with a syringe into venous blood collection tubes that were then centrifuged. Bacteria were separated at the surface of the gel and pellets were directly deposited on target MALDI plates. All plates were analyzed by MALDI-TOF MS and the microorganisms were identified by pattern matching with the libraries in BioTyper™ 2.0 software.
Results : *Escherichia coli* and *Pseudomonas aeruginosa* were used to evaluate colony to colony differences in the MALDI-TOF MS data. The scores were 2.371±0.044 and 2.377±0.039, respectively. *Prevotella buccae* and *Clostridium perfringens* were usedto investigate the influence of the culture time. The scores on day 1 and day 3 were 2.341 and 2.352, respectively**,** for *Prevotella buccae* and 2.435 and 2.411, respectively, for *Clostridium perfringens***.** The identification rates at the species and genus levels were 91.7% (429/468) and 97.0% (454/468) , respectively. By adding data for the clinical isolates to BioTyper™ 2.0, the identification rate increased. The identification rate was 86.5 % (32/37) at the species in cases with monomicrobial isolate in the positive blood culture bottle. *S. aureus* and *Staphylococcus haemolyticus* were identified in a case with polymicrobial isolate in the bottle, in which the polymicrobial isolate could not be identified simultaneously using MALDI-TOF MS methods.
Conclusions : MALDI-TOF MS is a rapid, simple and high-throughput proteomic technique for identification of bacterial species. Since colony to colony differences and effects of culture duration on the results are minimal, this method can be implemented in a conventional laboratory setting. For some pathogens, preanalytic processes should be refined and the current database requires improvement to obtain more accurate results. However, in general, MALDI-TOF MS performs equally well to conventional methods and is a promising technology for use in clinical laboratories.

PTu-073
11:10 – 12:20

Lectin-capturing and peptide affinity enrichment-based identification of cancer biomarker TIMP1 by high-resolution FT-ICR MS

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Keywords:
lectin-capturing, cancer biomarker, FT-ICR MS

Novel aspects:
TIMP 1 in human serum was identified to be a potent CRC biomarker by lectin-capturing and peptide affinity enrichment-based approach with MALDI FT-ICR MS.

Abstract:
Various aberrant glycosylation patterns have been known to be connected to cancer. Thus, an aberrant glycosylation state is important in studying pathological mechanisms involved in cancer or in developing cancer biomarkers. Cancer blood sample containing glycoproteins aberrantly glycosylated can be used as a target for development of a serological cancer marker. The lectin-capturing method has been widely used to fractionate protein glycoforms that have a specific glycan structure active to the lectin used. Therefore, this method is very attractive as a tool to capture a specific protein glycoforms of interest. Among a variety of lectins being employed for capturing an unique glycan form involving with cancer progression, L-PHA was used for capturing the aberrant glycoforms of glycoproteins up-regulated as a result of aberrant addition of β-1,6-N-acetylglucosamine (β-1,6-GlcNAc) by N-acetylglucosaminyltransferase-V (GnT-V), which is known to be up-regulated in invasive/metastatic colorectal cancer cells. By using lectin-coupled/FT-ICR MS with peptide affinity enrichment (SISCAPA) technique, it is investigated that a target protein, tissue inhibitor of metalloproteinase 1 (TIMP 1) can be a potent serological colorectal cancer marker. Since exact mass measurement of multiplex isotopic peaks of the target peptide surrogating glycoprotein TIMP 1 can be accomplished by virtue of high mass resolution, robust identification of the target peptide is achievable with 15 T FT-ICR MS. Whether TIMP 1 in human serum can be a potent CRC biomarker will be investigated by using FT-ICR MS. Additionally it is explored that high-resolution FT-ICR MS can be a useful tool toward identifying potential protein biomarker.

PTu-074
13:30 – 14:40

Relating ESI-MS response with polar-, structural- and primary-characteristics of neuropeptides: Developing a deeper understanding

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Keywords:
Neuropeptides, ESI-MS response, HDX MS

Novel aspects:
Reports exist on ESI response of small (m/z 300 to 1,600Da) or large proteins however, none on medium-sized molecules (1500 to 3500Da). Our research is aimed at filling this gap.

Abstract:
The search for key pain biomarkers is a hot topic within the pharmaceutical industry and it is clear from scientific literature that neuropeptides are implicated as potentially key markers. The detection and quantification of this class of peptides is best achieved using mass spectrometry (MS) but a deep understanding of how they ionise and behave in the gas phase is not yet fully understood. Currently, little is known about the factors affecting responsiveness of this size of peptides in positive electrospray ionization (ESI) MS though the intensities (selectivity and signal suppression) of the ions depends heavily on analyte basicity in solution, (p*K*b), sampling and instrumental conditions and on secondary structure. Other factors may be equally important and our research seeks to develop this understanding.

Neuropeptides are an important class of chemical messengers that modulate nervous system response. They act as neuronal messengers in the brain and central nervous system, influencing many neurobehavioral functions. Our main interest is in the neuropeptides that are relevant to the perception of pain, including substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and also corticotropim releasing factor (CRF) and prolactin releasing peptide (PrRP).

In positive ESI of peptides, multiply charged ions are typically produced. Multiple protonation is an important attribute in structure identification and accurate molecular weight determination in MS. To achieve protonation, the analytes must be basic both in solution (low pH) and in the gas phase. It is often expected that the primary sequence of the analytes and the p*K*b of the constituent ionisable amino groups of the peptide will be prominent factors in determining the number and intensity of protonation during the positive ESI response. However, this is not always the case [1,2]. In addition to the mass spectrometer settings, sample processing prior to the ESI stage is crucial to obtaining an adequate spectrum of ions and to achieving protonation. Our research indicates that the multiple protonation of specific neuropeptides cannot be controlled purely by varying pre-ESI parameters (pH of the mobile phase, flow rate (nano- and micro LC), concentration of organic modifier, peptide concentration), but that some charge states might be a result of the conformational state, peptide polarity and the position of very basic amino acids (arginine).

Here we present our investigation on the link between peptide characteristics and ESI response to allow robust quantitation of these important biomarkers especially during label-free quantitation. Factors investigated include peptide polarity, peptide conformation in solution (mobile phase) and the influence of the sequence position of specific amino acids (arginine), effects of electrolyte, and effect of conformation in solution (circular dichroism analysis and hydrogen-deuterium exchange HDX MS), and their detection in a saliva matrix.

(1) Daniel A. Abaye, Frank S. Pullen and Birthe V. Nielsen. Peptide polarity and the position of arginine as sources of selectivity during positive electrospray ionisation mass spectrometry (2011). Rapid Commun. Mass Spectrom. 25, p. 3597
(2) Daniel A. Abaye, Frank S. Pullen and Birthe V. Nielsen. Practical considerations in analysing neuropeptides, calcitonin generelated peptide and vasoactive intestinal peptide, by nanoelectrospray ionisation and quadrupole timeofflight mass spectrometry：monitoring multiple protonations (2011). Rapid Commun. Mass Spectrom. 25, p.1107

PTu-075
11:10 – 12:20

Fabrication of a titanium based MALDI bacterial chip for rapid, sensitive and direct analysis of pathogenic bacteria

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Keywords:
MALDI-MS, Biochip, mass sensor, pathogenic bacteria

Novel aspects:
We report a titanium chip for MALDI-MS produced from a simple, cost effective and rapid heat treatment process. It can be reused many times for direct analysis of pathogenic bacteria.

Abstract:
For the first time, we report the fabrication of a titanium bacterial chip for MALDI-MS produced from a simple, cost effective and rapid heat treatment process. This bacterial chip can be reused many times and is highly versatile. Besides, this bacterial chip can be applied for direct analysis of pathogenic bacteria. When immersed into a solution containing pathogenic bacteria, it is able to lead to rapid and sensitive capture of the pathogenic bacteria from the solution and hence act as a bacterial sensor. We have optimized the ideal chip which exhibits accelerated capture efficacy as the 800° C heat treated chip. These bacterial chips serve dual roles ; (1) They can be applied as MALDI-MS target plates for direct and highly sensitive bacterial analysis. (2) They can be used as bacterial sensors for direct analysis of the captured bacteria using MALDI-MS. The sensitivity of these chips when used as bacterial sensors is $< 10^3$cfu/mL. The lowest detectable concentration by direct MALDI-MS analysis was found to be 10^4 cfu/mL. The results were further justified by using standard plate counting method combined with Tukey Kramer statistical analysis and fluorescence imaging followed by image processing for fluorescence quantification using ImageJ software to substantiate the MALDI-MS results.

PTu-076
13:30 – 14:40

An optimized membrane proteins enrichment strategy for the bottom-up mass spectrometry approach

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Keywords:
Brain, Membrane proteins, Cloud point extraction, Two dimensional gel electrophoresis, Mass spectrometry

Novel aspects:
Application of the cloud point extraction and 2-DE separation to increase the coverage of membrane proteins by reducing the complexity of brain proteome for the mass spectrometry analysis.

Abstract:
Neuroproteomics is a continuously emerging field with the intention to identify biomarkers in various neurodegenerative disorders. The usage of mass spectrometry in neuroproteomics has become a powerful tool for the analysis of these very complex samples. However many challenges arise due to the diverse nature of the tissue and the high dynamic nature of the proteome. The complexity of biological samples can be reduced by introducing various pre-fractionation techniques during sample preparation, which determine the end result for the downstream proteomic analysis. With the aim to identify proteins in a highly complex brain tissue we tested various detergents, organic solvents and organic acids for the selective extraction of membrane bound proteins (MPs). The protocols where initially tested in mouse brain and the optimized extraction conditions were further applied to human brain tissue.

In this study, brain proteins extracted by various protocols were delipidated, on-filter digested and analyzed by reversed phase nanoliquid chromatography in combination with electrospray ionization tandem mass spectrometry using a 7 Thybrid LTQ-FT mass spectrometer. Detergent based lysis buffers produced high yield of proteins and high percentage of MPs (25-30%) which is in accordance with the genome expression levels. Among the tested detergents, Triton X-114 and n-Octyl-β-D-glucoside detergents provided the highest total number of identified proteins as well as the highest numbers and percentages of identified membrane (29%) and transmembrane proteins (52%). An additional advantage of Triton X-114 is temperature induced pre-fractionation separation called as cloud point extraction (CPE). In CPE technique using Triton X-114 when the temperature is raised above 36 °C, separation of hydrophilic and hydrophobic fractions was achieved. By exploiting this unique trait of polyoxyethylene non-ionic detergents, CPE was performed on human brain tissue to characterize the low abundant MPs as well as transmembrane proteins.

A total of 638 proteins were identified in the human brain extract, using a gel free on-filter tryptic digestion prior to mass spectrometry. All proteins were classified according to their sub-cellular localizations. Among them 30% were identified as MPs from which 20% are found exclusively in the hydrophobic fraction, 5% in the hydrophilic fraction and the remaining 5% in both. Hydrophilic and hydrophobic phases of the human brain CPE extracts were subjected to further separation by 2-DE and the protein spot patterns were analyzed. The hydrophobic membrane fraction shows unique spots in 2-DE that are typically masked by high abundant proteins in the hydrophilic fraction. The preliminary data suggests the potential application of pre-fractionation and separation steps that are compatible with "shotgun" based mass spectrometry approach to increase the coverage of low abundant MPs. The optimized extraction conditions mentioned here can be used for studying the potential biomarkers of interest in neurodegenerative disorders.

PTu-077
11:10 – 12:20

alpha-Tocopherol suppresses lipid peroxidation and behavioral impairment in mouse model of Down syndrome

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Keywords:
Ts65Dn mouse, Down syndrome, oxidative stress, lipid peroxidation, vitamin E

Novel aspects:
alpha-Tocopherol supplementation from an embryonic stage may be an effective treatment for the cognitive deficits associated with Down syndrome

Abstract:
It is widely accepted that oxidative stress is involved in the pathogenesis of Down syndrome, but the effectiveness of antioxidant treatment remains inconclusive. In the present study, we tested whether chronic administration of α-tocopherol, the biologically most active form of vitamin E, ameliorates the cognitive deficits exhibited by Ts65Dn mice, a mouse model of Down syndrome. Ts65Dn mice carry a segmental trisomy of mouse chromosome 16. Many of the genes in human chromosome 21 are conserved in mouse chromosome 16. Although Ts65Dn mice do not have all the features associated with Down syndrome (such as congenital heart defects), they display many Down syndrome-like features, including significant performance deficits in specific behavioral tasks. α-Tocopherol was administered to pregnant Ts65Dn females from the day of conception throughout the pregnancy, and to pups during their entire life, from birth to the end of the behavioral testing period. Lipid peroxidation products, including hydroxyoctadecadienoic acid, 7-hydroxycholesterol and 8-isoprostane were measured by using mass spectrometry. First, cognitive deficits were confirmed for Ts65Dn mice fed on the control diet, revealing reduced anxiety or regardlessness in the elevated plus maze task test and spatial learning deficit in the Morris water maze task test. However, supplementation with α-tocopherol attenuated both cognitive impairments. In addition, we found that the levels of 8-isoprostane in the brain tissue and hydroxyoctadecadienoic acid and 7-hydroxycholesterol in the plasma of Ts65Dn mice were higher than those in control mice. Supplementation with α-tocopherol decreased these lipid peroxidation products in Ts65Dn mice. Furthermore, we demonstrated that supplementation with α-tocopherol improved hypocellularity in the hippocampal dentate gyrus region of Ts65Dn mice. In addition, we found that the lipid peroxidation product acrolein was stained in the dentate gyrus region of Ts65Dn mice. These results indicate that lipid peroxidation closely relates to hypocellularity in the dentate gyrus region of Ts65Dn mice. These results imply that chronic α-tocopherol supplementation from the embryonic stage may be an effective treatment for the cognitive deficits associated with Down syndrome.

PTu-078
13:30 – 14:40

PGRN: a novel therapeutic target and biomarker for insulin resistance and obesity identified by differential proteome analysis

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Keywords:
insulin resistance, obesity, differential proteome analysis, diabetes, NBS method

Novel aspects:
Identification of a novel therapeutic target and biomarker for insulin resistance and obesity identified by differential proteome analysis.

Abstract:
<Background>
Insulin resistance is a characteristic feature of obesity and type 2 diabetes. Adipose tissue is now recognized as not only an energy-storage tissue, but also an endocrine tissue that secretes a variety of bioactive substances (adipokines) . Defects in adipokine secretion accompanying adipose tissue dysfunction contribute to the pathophysiology of insulin resistance and obesity. The relationship between inflammatory process and insulin resistance has recently drawn considerable attention. For example, TNF-alpha, a proinflammatory cytokine, has been shown to contribute to the development of insulin resistance. On the other hand, glucocorticoids, which are known to have an anti-inflammatory action, also induce insulin resistance in human and animals. Dexamethasone, a glucocorticoid, has been reported to impair insulin signaling and insulin-stimulated glucose uptake in adipose tissue, liver, and skeletal muscle. Since TNF-alpha and dexamethasone both induce insulin resistance despite their opposite inflammatory properties,we reasoned that there might be a common key mediator responsible for the cellular basis of insulin resistance induced by TNF-alpha and dexamethasone.
In the present study, we searched for a novel adipokine (s) that play a key role in developing insulin resistance using 3T3-L1 adipocytes treated with TNF-alpha or dexamethasone. For this purpose, we utilized a method of differential proteome analysis based on stable isotope labeling of proteins with chemical reagent 2-nitrobenzenesulfenyl chloride (NBSCl) incorporating six ^{13}C ($^{13}C_6$) or six ^{12}C ($^{13}C_0$) in the tryptophan residues.
<Aim>
The aim of this study is to search for a novel adipokine (s) that play a key role in developing insulin resistance.
<Methods>
To identify proteins associated with insulin resistance in adipocytes *in vitro*, differential proteome analysis using the NBS method was performed in 3T3-L1 adipocytes in which insulin resistance was induced by TNF-alpha or dexamethasone. The relative quantification and the identification of differentially expressed proteinswere performed using LC-MALDI-TOF MS.
<Results>
We found that 37 and 43 proteins were upregulated by TNF-alpha treatment and dexamethasone treatment, respectively, among which 21 proteins are common in the two treatments. We also found that 11 and 7 proteins were downregulated by TNF-alpha treatment and dexamethasone treatment, respectively, among which 2 proteins are common in these treatments. Identification of haptoglobin, serum amyloid A-3 (SAA3) protein precursor and nicotinamide phosphoribosyltransferase, all of which are known as the adipokines to be upregulated by such treatment, confirmed the validity of the method. After excluding known adipokines among the 23 proteins identified, we confirmed the results of differential proteome analysis on 8 proteins by immunoblot analysis using antibodies currently available. We finally selected progranulin (PGRN) because it is the only protein with both secretory and proinflammatory properties and further analyzed the protein functions.
PGRN was upregulated in both TNF-alpha-treated (1.66-fold vs. control) and dexamethasone-treated (3.01-fold vs. control) adipocytes. PGRN in blood and adipose tissues was markedly increased in obese mouse models and was normalized with treatment of pioglitazone, an insulin-sensitizing agent. Ablation of PGRN prevented mice from high fat diet (HFD) -induced insulin resistance, adipocyte hypertrophy, and obesity. PGRN deficiency blocked elevation of IL-6, an inflammatory cytokine, induced by HFD in blood and adipose tissues. Insulin resistance induced by chronic administration of PGRN was suppressed by neutralizing IL-6 *in vivo*.
<Conclusion>
PGRN is a key adipokine that mediates HFD-induced insulin resistance and obesity through production of IL-6 in adipose tissue, and may be a promising therapeutic target for obesity. Differential proteome analysis based on the NBS method described here should be useful for identifying proteins involved in insulin resistance in various tissues.

<Reference>
Matsubara T. et al., PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in adipose tissue. Cell Metab. (2012) 15, 38-50.

PTu-079
11:10 – 12:20

Direct molecular analysis by pico-drop sweat trapping from a single gland on a finger

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Keywords:
sweat, analgesic diagnosis, single sweat gland, live single-cell MS

Novel aspects:
Many compounds from a single sweat gland of a finger were successfully identified and drug kinetics in sweat was found to be similar to that in plasma.

Abstract:
Introduction
Development of diagnosis methods which is analgesic, non-invasive, highly sensitive and rapid is expected for future medicine. Since sweat includes biological and chemical compounds originated from blood, it reflects the concentration of these materials in blood. To prove sweat can be used for the analgesic diagnosis to monitor blood content such as drug, alcohol level, and also for doping, we picked a sweat droplet from a single sweat gland with a nanospray tip followed by direct submitting to nanoESI MS. We succeeded to achieve pico-drop sweat-nano mass spectrometry which enables direct sampling of sweat droplets of pico-lititer volume in analgesic way.

Method
We selected a finger's sweat gland as the region of picking sweat. The subjected volunteer humans were those who took medicine (troxipide, a stomach-medicine, KYORIN pharmaceutical Co., Ltd.) or not. First, we flushed over the surface of the finger and wiped water off with a tissue and then waited about 2 minutes until the temperature of finger surface recovered to the original temperature. Then we observed sweat from finger's sweat glands by a stereomicroscope, and sucked sweat quickly after secretion by a nanospray tip. We added 2 µL of ionization solvent of 70% MeCN aq from the back of the tip, then we analyzed that tiny amount of sweat by the mass spectrometer, LTQ-Orbitrap (Thermo Fisher Scientific) . The ion spray voltage was 1000V for positive ion detection mode. Aligned peaks were applied with PCA and t-test for sweat specific peak detection. We also tried to detect time-dependent change of the peak intensity of the medicine in sweat and compared to that of plasma concentration.

Result
We detected many peaks from a sweat droplet and identified. For example, urea, lactic acid, ornithine, urocanic acid (which is the metabolic intermediate of L-histidine) , sodium chloride, and 15 amino acids such as alanine, valine, proline, threonine, phenylalanine from the sweat of both subjects. The peak at *m/z* 295.1652 was also detected only in the drug-administered person. It was identified as troxipide by the exact mass and MS/MS. Sequential analysis of troxipide showed the highest intensity around 2 - 3 hours after taking medicine and the intensity lowered gradually. It was very similar to the previous report of plasma dynamics. We tried to calculate the concentration of this medicine in the sweat by making standard curve. First, we optimized the composition of ionizing solvent for sensitive detection of the drug. We prepared solvents with different blend ratio and analyzed the standard troxipide, and we found that 70% MeCN aq was the best. Next, we dissolved the standard medicine with the solvent and then analyzed. We found that the peak intensity was correlated highly with the concentration of medicine. The standard curve was linear between the concentration of 10nM to 1 µM with the correlation coefficient of 0.99, and the slope of the standard curve didn't change when we added sweat to troxipide solution. When we calculated troxipide concentration in sweat droplet with the standard curve, it was about 30nM. It was significantly lower than plasma concentration. These data suggest that molecular content can be quantitatively analyzed from directly isolated extremely tiny sweat using this method. If the analgesic diagnosis comes to be real by this method, it will greatly contribute the establishment of new clinical laboratory system.

PTu-080
13:30 – 14:40

Identifications of amyloidogenic proteins in FFPE tissue sections by MALDI-imaging coupled with on-tissue digestion and immunohistochemical/microscopic examinations

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Keywords:
MALDI-MS imaging, AL-type amyloidosis, On-tissue trypsin digestion, Immunoglobulin lambda

Novel aspects:
MALDI-imaging coupled with on-tissue digestion technique enables us to identify amyloidogenic proteins in FFPE sliced tissue sections without any procedure for retrieval of epitopes before on-tissue digestion.

Abstract:
Matrix-assisted laser desorption time-of-flight ionization (MALDI) -imaging MS (IMS) with MSMS analysis using *on-tissue* tryptic digests is a powerful tool for identification of disease-related proteins in formalin-fixed paraffin-embedded (FFPE) tissue sections. We applied these techniques to identify tryptic peptides of deposited amyloidogenic proteins in amyloidosis tissue sections. Sequence determination of tryptic peptides was performed using MALDI-MSMS analysis directly from Congo-red positive regions in sliced tissue sections without any procedure for retrieval of epitopes before *on-tissue* digestion.
A tryptic peptide, m/z=1073.5, was identified with the sequence, YASWYQQK, from 48th to 56th position of the Ig lambda chain. Heat-map image at m/z=1073.5 was overlapped with Congo red positive regions. Immunohistochemistry of FFPE tissue sections were confirmed to react with anti-amyloid P component and anti-lambda chain antibodies, not an anti-serum amyloid A antibody. From results of IMS analysis and immunohistochemistry, we diagnosed AL-type of lung-amyloidosis in this case. We also identified amyloidogenic proteins at the other 2 cases of amyloidoses. In conclusion, the protocol enables us to identify insoluble deposited proteins in amyloid plaques using FFPE in the same way as fresh frozen tissues.

PTu-081
11:10 – 12:20

LC-MS/MS based basal and ACTH-stimulated serum concentrations of adrenal and gonadal steroid homones and its application on heterozygote CYP21A2 mutations

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Keywords:
adrenal and gondal steroid concetrations, heterozygote carriers of 21OHD, ACTH stimulation test

Novel aspects:
Multisteroid assay for basal and ACTH stimulated steroid hormones, identifiying heterozogote carriers for 21-hydroxylase deficiency

Abstract:

Background:
Congenital Adrenal Hyperplasia (CAH) is composed of several inherited defects of steroid biosynthesis that impair cortisol biosynthesis. 21-hydroxylase deficiency (21OHD) is the most common form of CAH and has a high prevalence of asymptomatic heterozygote carriers in the general population. A screening method and case detection of heterozygote carriers of 21OHD is therefore greatly useful. The key steroid of 21OHD is 17-hydroxyprogesterone, which is highly elevated in homozygote carriers while heterozygote carriers have normal to slightly elevated values. Both derivatives 11-deoxycortisol and cortisol are humiliated in 21OHD.

Objective:
To determine basal and ACTH-stimulated values of progesterone, deoxycorticosterone (DOC) , corticosterone,17-hydroxyprogesterone (17OHP) , 11-deoxycortisol (11S) , 21-deoxycortisol (21S) , cortisol (F) , cortisone, androstenedione, testosterone and dihydrotestosterone genotypic normal subjects and heterozygote carriers of CYP21A 2 mutations, to generate reference ranges for these hormones, and to determine a distinctive basal or ACTH-stimulated parameter for separation of heterozygote carriers of CYP21A 2 mutations from normal controls.

Methods and Materials:
We established an assay for measuring all hormones in small sample volume. 0.1 mL plasma was extracted by solid phase extraction (SPE) and analyzed using an UPLC-MS/MS in MRM mode. We compared our assay to different commercial RIA assays and a RIA with prior Extraction.
The study included 58 heterozygote carriers of CAH (35 males, 23 females, age range 6 -78 years) and 44 control subjects (25 males, 19 females, age range 8 -58 years) . For all probands basal steroid hormone values, ACTH-stimulated values after 30 minutes and after 60 minutes were determined. Quotients of 17OHP+21S/F and 17OHP/11S were calculated.

Results:
The coefficients of determination for the comparison were between 0.82 for DOC RIA *vs* LCMSMS and 0.99 for 17OHP RIA *vs* LCMSMS. With this validated assay basal and ACTH stimulated steroid concentrations for controls and heterozygote carriers were determined. Basal steroid concentrations in controls were 0.06-2.84 ng/mL (17OHP) , 0.09-1.2 ng/mL (11S) and 40-370 ng/mL (F) . ACTH-stimulated values after 30 min in controls were 0.06-2.94 ng/mL (17OHP) , 0.09-1.8 ng/mL (11S) , and 40-474 ng/mL (F) . The ACTH-stimulated values after 60 min were 0.09-2.81 ng/mL, (17OHP) , 0.09-2.3 ng/mL (11S) and 100-534 ng/mL (F) . We found significantly lower basal values for F in heterozygote carriers ($p<0.003$) . The ACTH-stimulated values of 17OPH after 30 min and 60 min were significantly higher in heterozygote carriers ($P<0.001$) . The quotient 17OHP/11S was significantly higher in heterozygote carries both, at baseline as well as after ACTH-stimulation ($p<0.001$) . The quotient 17OHP/F was significantly higher in heterozygote carriers after ACTH-stimulation ($p<0.1$) . However all values showed an overlap between heterozygote carriers and normal control subjects.

Conclusion:
We determined basal and ACTH reference ranges. We could distinguish between heterozygote carriers of CYP21A 2 mutations and normal controls on the basis of the stimulated 17OHP and the quotient of 17OHP+21/11S with a specificity and sensitivity of 90%.

PTu-082
13:30 – 14:40

Discovery of novel urinary biomarker candidates for diagnosis of prostate cancer

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Keywords:
MALDI-TOF/MS, prostate cancer, urine, peptidomics, biomarker candidates.

Novel aspects:
We discovered novel urinary biomarker candidates for diagnosis of prostate cancer. The amino acid sequences of the candidates were identified using a MALDI-DIT-TOF/MS.

Abstract:
Background: For men in the United States, prostate cancer (PCa) is the most common type of cancer and the second highest cause of cancer death [1]. In Japan, recently, mortality rates for PCa have been increasing dramatically as well [2]. Large-scale clinical detection of prostate specific antigen (PSA) levels in serum as a biomarker of PCa has been carried out since the 1990s [3]. While the over all benefits and risks of population PSA screening for PCa continues to be assessed, the problem with PSA not being cancer specific marker will continue to be a clinical problem [3,4]. For detecting PCa, the serum PSA cutoff value of 4.1 ng/mL is used globally, yielding only a sensitivity of 20.5% though a specificity of 93.8% [5]. Therefore, it is supposed that there is no cutoff value of PSA with both high sensitivity and high specificity for monitoring healthy men for PCa, but rather a continuum of PCa risk at all values of serum PSA. Therefore, great emphasis has been placed on the need to discover novel biomarkers for diagnosis of PCa. We focused on urine samples voided after prostate massage, because the urine samples after prostate massage could be expected to contain many peptides and protein-fragments secreted from prostatic microenvironments, enabling detection of secreted prostate products as potential sources of PCa specific biomarkers [6]. Therefore, we conducted peptidomic and proteomic analyses of the samples using a matrix assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF/MS) in order to discover novel biomarkers for diagnosis of PCa.
Methods: This study was conducted with the permission of the Ethics Committee of Graduate School of Medicine, Kyoto University. All examinations were performed with the informed consent of all subjects. Urine samples were collected as follows : the clinician performed a digital rectal exam and gently massaged each side of the prostate gland three times ; and the prostate massages stimulated release of prostate fluids into the urethra. The prostate fluids were collected when the subjects voided urine following the massage. The collected samples were filtrated with 100 μm cell strainers and were centrifuged at 2,000 x g for 10 min at 20&#8451 ; to exclude urinary debris. After centrifugation, the supernatants were filtrated with the strainers again. The filtrated supernatants were stored at -80&#8451 ; until the analyses. In this study, we developed a new analytical system : urinary peptides and protein-fragments captured by ion-exchange resin were applied to the analyses using a MALDI-TOF/MS. This system was composed of ion-exchange capturing and concentrating processes of them in the urine samples, and of a desalting of the concentrated biomolecules. CM-Sepharose as an ion-exchange resin and home-built MALDI digital ion trap (DIT) TOF/MS^n manufactured by Shimadzu Corporation were utilized. Differential analyses of mass profile data between the two groups, PCa samples and non-cancer samples, were performed with a reflectron pass-through mode of MALDI-DIT-TOF/MS. Identification of peaks was analyzed with an ion-trap mode of the TOF/MS^2. The peak lists obtained from the MS^2 spectra were used to identify amino acid sequences using the Mascot search engine. We targeted intact peptides and protein-fragments between 1,000 and 5,000 Da in the urine samples, because the sequences of many of them can be confirmed using MALDI-DIT-TOF/MS^n in this molecular weight range.
Results and Discussion: Novel urinary biomarker candidates were discovered for diagnosis of PCa. In this conference presentation, we would like to discuss the relationships between these candidates and the diagnostic possibilities of PCa.
References: [1] Urol Clin North Am 2002 ; 29 : 173. [2] Jpn J Clin Oncol 2005 ; 35 : 690. [3] J Proteomics 2009 ; 72 : 907. [4] N Engl J Med 2001 ; 344 : 1373. [5] JAMA 2005 ; 294 : 70. [6] World J Urol 2007 ; 25 : 557.

PTu-083
11:10 – 12:20

Mass spectrometric determination of carcinoid syndrome tumor marker 5-HIAA in human serum

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Keywords:
5-hydroxyindole acetic acid, Carcinoid syndrome, Diagnostics

Novel aspects:
A HPLC-electrochemical detection assay is usually used to monitor the disease. We developed a simple and sensitive LC-MS/MS method for the quantification of serum 5-HIAA.

Abstract:
Aim:

In patients with carcinoid disease, urinary 5-hydroxyindole acetic acid (5-HIAA) is generally used to diagnose and monitor the disease. To avoid the cumbersome 24h-urine collection, we developed and validated a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of serum 5-HIAA.

Materials and Methods:

We used serum samples from healthy volunteers. Samples were spiked with 5-HIAA-D_2 internal standard and extracted using WAX µElution plates (Waters) .The assay was performed using a Kinetex C18 column (Phenomenex) coupled to an Agilent Technologies 1200 HPLC and an AB Sciex 4000 QTRAP MS with an ESI ion source in positive ionization mode. The duration of one run was 3 min.

Results and Discussion:

The assay was sensitive with a limit of detection of 7.8 nmol/l and limit of quantification of 9.5 nmol/l and had a wide assay range of 10-5000 nmol/l. The within-assay variation was 3.8-5.3% and the day-to-day variation 5.8-10.5%. When 5-HIAA was added to three serum samples containing 99-252 nmol/l endogenous 5-HIAA, the mean recovery was 90 % (spiked with 200 nmol/l) and 97 % (spiked with 500 nmol/l) showing good accuracy.

Conclusions:

We have developed a sensitive and accurate LC-MS/MS method for determination of serum 5-HIAA. The assay has a short run-time and a wide assay range. Compared to 24h-urine collection, blood sampling is well controlled and fast. Therefore, this assay may provide faster and more accurate diagnosis and follow-up of carsinoid syndrome as compared to determination of urinary 5-HIAA.

PTu-084
13:30 – 14:40

Chip-based electrospray ionization tandem mass spectrometry a novel tool for rapid diagnostic of Fabry disease

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Keywords:
Fabry disease ; chip-based nanoESI ; CID ; ETD ; rapid diagnosis

Novel aspects:
We developed here a novel platform for rapid and reliable diagnosis of Fabry disease based on chip-nanoESI CID and ETD multistage MS in combination with enzyme assay.

Abstract:
Fabry disease is an inherited condition belonging to the group of lysosomal storage diseases (LSDs) characterized by the absence or reduction of alpha-galactosidase A activity in lysosomes, that results in accumulation of globotriaosylceramide (Gb 3) and related neutral glycosphingolipids in blood vessel walls. The clinical onset occurs in childhood and over time, microvascular affliction of the kidneys, heart, and brain progresses, leading to early death. One of the major problems in the management of Fabry disease is the lack of a standardized protocol for early diagnosis, before a major deterioration of the tissues to arise. Therefore, in the last years important efforts were invested in development of methods for reliable detection of the disease. In terms of rapid diagnosis, Fabry disease remains to date one of the LSDs, which benefited the most from the advantages of mass spectrometry (MS) . The current MS-based strategies [1,2] target either the detection of non-degraded Gb 3 and lyso-Gb 3 in plasma, urine and tissue extracts or monitoring the alpha-galactosidase activity from dried blood spots (DBS) .
Although capable to provide early detection of the disease, occasionally, DBS-MS approaches generated false negative results. A cause of these errors is the lack of MS systems able to ensure the level of reproducibility necessary for comparative screening patient vs. healthy control. A reduced sensitivity and throughput of the MS experiments as well as the carry-over from sample to sample which might occur during successive MS analyses could also influence the obtained data. To eliminate these drawbacks, we propose here a novel platform for reliable diagnostic of Fabry disease, based on enzyme assay and fully automated chip-nanoelectrospray (nanoESI) MS, collision-induced dissociation (CID) and electron transfer dissociation (ETD) multistage MS (MS^n) performed on a high capacity ion trap (HCT) MS, in-laboratory coupled to a NanoMate 400 robot. In our approach the enzyme assay products derived from DBS of 11 healthy donors and two Fabry disease patients were automatically infused by NanoMate robot into HCT MS, in a high throughput regime, under identical conditions. The cleavage of substrate GLA-S generated a product, GLA-P, which could be quantified related to an internal standard GLA-IS. Due to the high sensitivity and reproducibility of the experiments provided by chip-nanoESI MS, the comparative analysis patient vs. control indicated a 13 fold reduction in GLA-P : GLA-IS ratio in the case of the subjects suffering from Fabry disease. GLA-P : GLA-IS ratios calculated according to the absolute intensity of the MS signals showed 13 times lower relative values in Fabry patients as compared to controls, which clearly indicates the dissimilarity in enzyme occurrence or activity. To substantiate this diagnostic, GLA-P and GLA-IS corresponding ions were confirmed by CID and ETD used complementarily. This first employment of ETD in rapid diagnosis of a LSD was possible due to the ability of chip-nanoESI to form multiply charged cations. The high performance of NanoMate-HCT MS^n in terms of sensitivity and reproducibility was also crucial in discriminating the Fabry cases where alpha-galactosidase is completely absent from those in which the enzyme is present at the trace level. Additionally, the method demonstrated the capability to distinguish the Fabry patients unable to synthesize the alpha-galactosidase from those producing an inactive enzyme. Both aspects have a major clinical importance for adequate therapy orientation. These results recommend chip-nanoESI MS as a valuable tool for detection and monitoring of rare diseases.

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PTu-085
11:10 – 12:20

Performance Evaluation of an Analytical LC-MS/MS Method for Accurate Measurement of 25-OH Vitamin-D in Serum

Heather Gamble, Changtong Hao, Sha Joshua Ye, George Scott

IONICS Mass Spectrometry, Toronto, Canada

Keywords:
LC-MS/MS, Vitamin D, New Methods

Novel aspects:
Development of a rapid, highly sensitive method for quantitative analysis of 25 (OH) vitamin D in human serum with limited sample preparation requirements.

Abstract:

Background

Vitamin D is a group of fat-soluble hormones. The metabolites of vitamin D have a critical physiological function in maintaining calcium and phosphate homeostasis. Vitamin D deficiency can best be diagnosed using 25 (OH) vitamin D as opposed to the other vitamin D metabolites because 25 (OH) vitamin D levels in serum reflect the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency. We have developed a fast and accurate LC-MS/MS method to simultaneously measure 25 (OH) vitamin D 3 and 25 (OH) vitamin D 2 covering an analytical concentration range of 0.1-100 and 0.4-125 ng/mL, respectively.

Methods

25 (OH) vitamin D was purchased from Sigma (Milwaukee, WI) and vitamin D free human serum was purchased from Golden Western Biologicals (Temecula, CA) . The 25 (OH) vitamin D spiked serum was deproteinized by mixing one volume of serum, one volume of 0.1% formic acid solution and two volumes of acetonitrile. The mixture was vortexed for one minute followed by centrifugation for 15 min. The supernatant was transferred to a clean vial for quantitation by LC-MS/MS. Two µL of supernatant were loaded on a Fortis C18 column (50x2.1mm, 3 µ) coupled to an IONICS 3 Q 200 Series triple quadrupole detector with a Shimadzu Prominence UPLC system. The binary gradient program used to elute 25 (OH) vitamin D at a flow rate of 0.4 mL/min was as follows : starts at 95% A (water : methanol = 95 : 5, v/v, with 0.1% formic acid and 5 mM ammonium acetate) ; at 0.01 min, changes to 85% B (water : methanol = 5 : 95, v/v, with 0.1% formic acid and 5 mM ammonium acetate) ; at 0.3 min, increases to 100 % B at 3 min, holds for 1 min, then decreases to 5 % B and equilibrates the column for two minutes. The quantifier mass transitions used for 25 (OH) vitamin D 3 and 25 (OH) vitamin D 2 are 401.3/257.2 and 413.2/355.2, respectively. All the solvents used in this method are HPLC grade.

Results

This method covers a concentration range of 3 orders of magnitude from 0.1and 0.4 ng/mL for 25 (OH) vitamin D 3 and 25 (OH) vitamin D2, respectively, while maintaining excellent linearity ($R^2 = 0.998$) . The intra-day and inter-day variabilities for both 25 (OH) vitamin D range from 1 to 10%. The intra-day variability is determined by processing 4 replicates of each QC sample and the inter-day variability is determined with 4 replicates in 3 batches.

Conclusion

A 6-min, sensitive, and reliable LC-MS/MS method was developed for quantitative determination of 25 (OH) vitamin D in human serum. The sample preparation for this LC-MS/MS method is simple and well suited for routine clinical analysis of 25 (OH) vitamin D.

PTu-086
13:30 – 14:40

Phosphoproteomic analysis of an EGFR/HER2 targeted drug-resistant gastric cancer cell line

Yong Yook Lee[1], Minjueng Kang[1], Byoung-Kyu Cho[1], Hwang-Phill Kim[2], Tae-You Kim[2], Eugene C Yi[1]

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Keywords:
Lapatinib, gastric cancer cell line, SUN216, phosphoprotein, LC-MS

Novel aspects:
Studies were performed with Q-Exactive LC-MS tandem mass spectrometry in lapatinib resistant gastric cancer cell line for a quantitative phosphoproteome evaluation.

Abstract:
Lapatinib, a dual kinase inhibitor of EGFR and HER 2 tyrosine kinases, has shown promising *in vitro* results in inhibiting the growth of HER 2 -overexpressing cancer cells. However, like other EGFR-targeted drugs, lapatinib drug resistance remains a major clinical challenge in targeted cancer therapy due to poor understanding of underlying mechanisms. To study the cellular mechanisms of the targeted cancer drug resistance, we generated lapatinib-resistant HER 2 -overexpressing human gastric cancer cell line (SNU216 LR) incubating lapatinib-sensitive SNU216 cells for a prolonged period with lapatinib. To identify signaling networks that are crucial for maintaining the acquired drug resistance in SNU216 LR, we performed a quantitative phosphoproteome evaluation for the SNU216 LR cells using a phosphopeptide enrichment method followed by Q-Exactive LC-MS tandem mass spectrometry analysis. Several phosphoproteins involved in the downstream signal transduction of Ras/Raf/mitogen-activated protein kinase (MAPK) pathway were found to be upregulated in SNU216 LR compared to its the parental cell line. These data suggest that activation of the Ras/Raf/MAPK signaling pathway could be a potential mediator of resistance to lapatinib.

PTu-087
11:10 – 12:20

A Fast Method for Quantitative Determination of Methotrexate in Human Serum by a LC-MS/MS

Sha Joshua Ye, Heather Gamble, George Scott

IONICS Mass Spectrometry, Toronto, Canada

Keywords:
Drug Monitoring, New Methods, LC-MS/MS

Novel aspects:
Development of a rapid, sensitive, accurate LC-MS/MS method for quantitation of methorexate in serum requiring little sample preparation.

Abstract:

Background

In the present study, an LC-MS/MS method was developed and verified for quantitation of methotrexate in human serum. Methotrexate, a classical antifolate drug which has been in use for many years, is one of the oldest chemotherapy drugs. It is effective in treating various neoplastic diseases such as acute leukemia, non-Hodgkin lymphoma, breast carcinoma, and more recently autoimmune disease at much lower concentrations. Fast LC-MS/MS with high selectivity and sensitivity is a preferred analysis technique.

Methods

Methotrexate and human serum were purchased from Sigma (Milwaukee, WI). The methotrexate spiked serum was cleaned by protein precipitation by mixing one volume of serum, one volume of 0.1% formic acid solution and two volumes of acetonitrile. The mixture was vortexed for one minute followed by centrifugation for 15 min. The supernatant was transferred to a clean vial for quantitation by LC-MS/MS. Five µL of supernatant were loaded on an Imtakt C 8 column (75x2.0mm, 3 µm) coupled to an IONICS 3 Q 200 Series triple quadrupole detector with a Shimadzu Prominence UPLC system. The mass transitions of 455.1/134.0 and 455.1/308.0 are used for methotrexate quantitation. The total LC cycle time is 3 min at a liquid flow rate of 0.4 mL/min. All the solvents used in this method are HPLC grade.

Results

This method covers a concentration range of 0.06 to 275 µmol/L while maintaining excellent linearity ($R^2 = 0.999$). The intra and inter-day variability of three level QCs for this experiment were < 7 and 8 %, respectively. The intra-day variability is determined by processing 4 replicates of each QC sample and the inter-day variability is determined with 4 replicates in 3 batches.

Conclusion

A fast, sensitive, and accurate LC-MS/MS method was developed for methotrexate in serum. The LLOQ achieved is 0.06 µmol/L with 5 µL sample injection. This LC-MS/MS method requires little sample preparation and is well-suited for routine therapeutic drug monitoring of methotrexate.

PTu-088 Chip Based MicroLC-MS for Small Molecules Analysis

13:30 – 14:40

Remco van Soest[1], Xiang Zhu[1], Xiaohong Chen[2], Nicole Hebert[1], Hua-Fen Liu[2]

[1]Eksigent/AB SCIEX, [2]AB SCIEX

Keywords:
nanoLC chips chromatography steroids lc-ms

Novel aspects:
Use of chips in nanoLC-MS for analysis of small molecules with increased sensitivity.

Abstract:
Sensitive nanoLC-MS is widely used in a variety of research areas. Although providing excellent sensitivity, the low flow rate (200-300 nl/min) reduces sample throughput which is important for small molecule analysis in clinical research. 200 um ID chip based columns at proportionally higher flow rates (1.5 uL /min) were used to perform small molecule analysis. The delay time can be greatly reduced compared to nanoLC while still providing higher sensitivity with smaller sample volumes than conventional HPLC methods. In this study the applicability of chip based microLC-MS for the analysis of small molecules (panel of steroids) in complex biological matrices) is investigated.

The system used consisted of a NanoLC-Ultra (Eksigent, part of AB SCIEX) nanoLC system, cHiPLC Nanoflex and a QTRAP 5500 (AB SCIEX) mass spectrometer. Separation was performed on a 200 um x 5 cm C18 chip column. Mobile phase A is H_2O (0.1% formic acid) and mobile phase B is ACN (0.1% formic acid) . Flow rate is 1.5 uL/min and the gradient is 25%-60% B in 6 min.
Serum was spiked with a standard panel of steroids. After protein precipitation 1 uL of supernatant was injected. Data shows high reproducibility across all runs. Retention time RSD is less than 0.5%, allowing for scheduling MRM's with a narrow retention time window. Good linearity was observed for the calibration curves of all analytes with correlation coefficients better than 0.99. Observed LOQ's are improved over typically reported LOQ's in the literature using conventional LC/MS, taking into account injected amount of sample.

PTu-089 Accurate Mass LC-MS/MS Profiling of Synthetic Cannabinoids

11:10 – 12:20

Benjamin J Figard[1], Christopher L Pennington[2], Jeff Dahl[3], Susan Leonard[4], Jorge R Smith[1]

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Keywords:
Accurate Mass Profiling Cannabinoids

Novel aspects:
Accurate Mass Profiling by LC-MS/MS for Sythetic Cannabinoids and comparison to Neutral Loss and MRM methodolies for LCMS QQQ.

Abstract:
Designer drugs are typified by substances such as ecstasy, bath salts, and synthetic cannabinoids. Many designer drugs are perceived as trendy, and cutting edge, with the most successful ones, such as MDMA, becoming mainstream. Rapidly changing formulations and the high prevalence of related analogues makes tracking and stopping designer drug usage problematic for law enforcement. In many cases the compounds in designer drugs are structurally related to, and just as potent as, illegal/regulated compounds, but are themselves, "legal. "
In emergency rooms the rapid changing nature of designer drugs present a problem to clinicians attempting to treat individuals under their influence. In many cases available screening methods are not capable of identifying what an individual has consumed. As a result clinicians are required to rely on the account of the patients who, in many cases, are too impaired to remember , or do not know, what they took. In an effort to better understand the complex, changing nature, of designer drugs LC MS/MS analysis has been applied to characterize the components of bath salts and synthetic cannabinoids.

PTu-090 A simple GC/MS procedure for vitamin D metabolite analysis

13:30 – 14:40

Anna M Przyborowska[1], Graham D Carter[2], Julia C Jones[2], John M Halket[1,2,3]

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Keywords:

Vitamin D metabolites；GC/MS；Isotope-dilution；Serum；Derivatization

Novel aspects:

Simple and relatively inexpensive GC/MS procedure for analysis of important vitamin D metabolites in human serum

Abstract:

The level of 25-hydroxy-vitamin D3 in serum is universally used as an indicator of vitamin D status, a parameter of increasing importance in many aspects of health, including osteoporosis, cancer and, recently, multiple sclerosis. Although GC/MS methods have been developed, they have been considered too complex for routine use as they involve a derivatization [1] or dehydration [2] step. Immunoassay, HPLC and LC/MS/MS methods are widespread. In the current work, GC/MS has been revisited and a simplified procedure developed using a relatively inexpensive mid-range GC/MS system as commonly found in clinical laboratories. With superior chromatography as well as high specificity, the isotope-dilution procedure has potential as a standard reference method available to DEQAS (http：//deqas.org/), the external quality assessment scheme for vitamin D metabolites, in order to assess the validity of using the All-Laboratory Trimmed Mean (ALTM) as a surrogate for the "true" value.

Calibrators, blank, spiked serum and patient serum samples (1 ml spiked with d6-25-hydroxy-vitamin D3 as internal standard) are deproteinized with acetonitrile and purified by a solid phase extraction procedure (OASIS HLB 1 cc) cartridges. After elution with acetonitrile/methanol, trimethylsilyl ether derivatives are formed by reaction of dry residues with BSTFA/pyridine/acetonitrile. Samples are analyzed by GC/MS with data acquired in the SIM mode：m/z 439, 413 and 544 for the pyro forms of 25-OH-Vitamin D3 and m/z 445 and 419 for the internal standard.

The trimethylsilyl ether derivatives of 25-OH-Vitamin D3 and the deuterated internal standard show good gas chromatographic and mass spectrometric properties. The common interferent in some LC/MS/MS assays, 3-epi-25-hydroxy-vitamin D3, is well separated.

The method is linear in the range 0-75 ng/ml. Accuracy and precision data are reported together with validation data and results of spiking experiments as well as data from a range of DEQAS quality assurance samples analyzed. The method currently has a higher limit of detection for the vitamin D2 analogue, used as a supplement in some countries. Although it is not suggested as an alternative procedure for the determination of vitamin D status in patients, it should prove useful for the rapid investigation of any questionable results obtained from routine assay procedures.

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PTu-091
11:10 – 12:20

Highly sensitive method for arginine vasopressin in human plasma determined by LC-MS/MS

Shin-ichiro Nitta[1], Yasuko Tsukazaki[1], Naoto Senda[1,5], Shigeru Yamada[2], Kazunobu Yamamoto[2], Mark M Garner[3], Hesham Ghobarah[3], Kunihiko Koriyama[4], Kinya Kubo[5]

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Keywords:
Arginine vasopressin, Vasopressin, LC-MS/MS, Hybrid capillary

Novel aspects:
High sensitive determine of arginine vasopressin in plasma was developed by LC-MS/MS equipped with a "metal and fused silica hybrid capillary ".

Abstract:

Introduction
Arginine vasopressin (AVP) is one of peptide hormones which is synthesized in the hypothalamus and is stored in the posterior pituitary. Most of them stored in the posterior pituitary is secreted to the blood stream. The regulation of the amount of water in the body is the most important role of AVP. AVP increases urine osmolarity, increase of urine contents, and decreases the excretion of water. Furthermore, AVP increases the vasoconstriction of peripheral blood vessel which leads to the arteriopressor. The reduction of AVP excretion or that of sensitivity on AVP in the kidney leads to the diabetes insipidus. It also shows hypernatremia (the increase of sodium concentration in plasma) , polyuria (over production of urine) and polydipsia (thirst) . On the other hand, the hyperexcretion of AVP (syndrome of inappropriate antidiuretic hormone, SIADH) cause hyponatremia. We developed and validated the analytical method of AVP level in human plasma using the hybrid triple quadrapole LC-MS/MS system equipped with a "metal and fused silica hybrid capillary " as electrode.

Method
The standard solution of AVP (1 to 400 pg/mL, 7 points) was prepared with 1% acetic acid aqueous solution containing 5% acetonitrile. 400 μL of control serum (CONSERA, NISSUI PHARMACEUTICAL CO., LTD. Tokyo, Japan) was mixed with 40 μL of each standard solution to prepare for the calibration samples. Calibration/human plasma samples were extracted with solid phase extraction (OASIS HLB, Waters Corporation, MA, USA) . After extracted sample was reconstituted by 200 μL of 1% acetic acid aqueous solution containing 5% acetonitrile. LC-MS/MS was couple with conventional HPLC system. A mobile phase were through a Inertsil Sustain C8 column (flow rate : 100 μL/min, 100 × 2.1 mm ID, GL Science, Tokyo, Japan) using the gradient program. Mobile phase was used 0.02% acetic acid aqueous solution as line A and acetonitrile as line B. The electrode of this LC-MS/MS system was change from 100 μm diameter to 25 μm diameter.

Preliminary
We developed a high sensitive analytical condition (1 pg/mL of LLOQ) on AVP in human plasma using a LC-MS/MS system. Linearity of the calibration curve was ranged from 1 to 400 pg/mL. In the calibration range, the precision (15.0% and lower) and the accuracy (±15.0% and lower) were acceptable. Our method was very useful to detect AVP levels in human plasma collected from 6 volunteers except for a part of analytical samples, which were collected from several other volunteers because of lower than LLOQ (about 0.3 to 0.8 pg/mL) .

PTu-092
13:30 – 14:40

More and Less Susceptible Amino Acid Residues to In-source Decay of Protein and Peptide in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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Yokohama City University, Yokohama, Japan

Keywords:
ISD, MALDI, Susceptibility, Protein, Peptide

Novel aspects:
More and less susceptible amino acid residues to MALDI-ISD of protein and peptide have been determined from the stand points of secondary structure of protein.

Abstract:
In-source decay (ISD) coupled with MALDI has been recognized as a top-down approach for obtaining internal amino acid sequence from intact protein without any pre-digestion. Further, MALDI-ISD is a useful method for the analysis of post-translational modification, because the method results in specific cleavage at N-Ca bond on the peptide backbone without loss or degradation of amino acid side chains and modified groups such as phosphate group. The MALDI-ISD uses hydrogen radical transfer from matrix to analyte molecules in principle. The hydrogen radicals produced from active hydrogens of hydroxyl or amino groups in aromatic matrix molecules attach to carbonyl oxygen on the backbone, and peptide or protein radical ion species are generated with protonation or deprotonation. The resulting radical ion species degrade within several ten nanoseconds in the MALDI ion source, leading to the formation of N-terminal side c-series and C-terminal side z-series ions.

In this report, the susceptibility of the N-Ca bond oo the peptide backbone to specific cleavage by MALDI-ISD has been studied from the standpoints of amino acid residues and secondary structure of protein. Analytes used for this purpose were horse cytocrome c (*M*r 12360) , horse myoglobin (*M*r 16951.4) , bovine serum albumin (*M*r 66402.8) , cucumber green mottle mosaic virus court protein (*M*r 17305.3) , and a tetra-phosphorylated model peptide (*M*r 3606.6) . In order to determine more and less susceptible amino acid residues to the ISD, further MALDI-ISD spectra reported from other research groups so far were used. From the peak abundance of resulting c-series ions in MALDI-ISD spectra of protein and peptide, it was concluded that more susceptible amino acid residues to MALDI-ISD than other residues were Xxx-Asp, Xxx-Asn, Gly-Xxx, Val-Xxx and Ile-Xxx bonds, while less susceptible residues were Xxx-Gly, Xxx-Val and Xxx-Ile bonds. Further, the resulting c-series ions originating from the cleavage at N-Ca bonds in flexible secondary structures such as turn and bend, which are free from intra-molecular hydrogen-bonded helix structure, gave relatively intense peaks. This is in agreement with the observation that Gly, Asp and Asn residues are preferred in turns rather than helix.

It has been reported that amino acid residues have preferences for certain secondary structures such as helix, sheet, turn, bend or isolated residues. The turn, bend and isolated residue are classified as flexible regions without intra-molecular hydrogen-bonding as seen with helix and sheet, so that the carbonyl oxygen atoms on the backbone in the flexible regions are able to interact with or bind to matrix molecules and hydrogen radicals. In contrast, helix and sheet structures protect the carbonyl oxygen atoms from the interaction with matrix molecules and hydrogen radicals. The amino acid residues Glu, Ala, Leu, Met, Gln and Lys are preferred in the hydrogen-bonded secondary structure of helix, while Pro, Gly, Ser, Cys and Tyr residues rather tend to inhibit formation of helix. In contrast, Gly, Asn, Asp, Pro and Ser are rather preferred in the flexible secondary structure of turn. It is expected from the trend described above that the amino acid residues susceptible to MALDI-ISD, such as Gly-Xxx, Xxx-Asp and Xxx-Asn, are preferred in turn or other flexible secondary structures, although the N-Ca bond of Xxx-Gly residue is less susceptible.

The results obtained from the MALDI-ISD of proteins and peptides here show a trend whereby relatively flexible regions such as turn and bend structures are more susceptible to MALDI-ISD than intra-molecular hydrogen-bonded secondary structures such as helix. This suggests that protein molecules embedded into matrix crystal still maintain the secondary structures as determined by X-ray crystallography. The mechanism for more susceptible to MALDI-ISD in Gly-Xxx, Xxx-Asp and Xxx-Asn bonds will be discussed.

PTu-093 New MALDI matrices for negative-mode metabolomics

11:10 – 12:20

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Keywords:
MALDI, metabolomics, matrix, negative-mode

Novel aspects:
Discovery of matrices for negative-mode MALDI of low-mass compounds (especially metabolites) with higher sensitivity and/or better versatility than currently known matrices.

Abstract:
Quantitative detection of a wide range of metabolites is a prerequisite to meaningful metabolomic studies. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been shown to be a useful method for this task, with certain advantages over electrospray-based measurements [1]: high sensitivity, high tolerance for salts and contaminants, and a generally straightforward sample preparation.
Matrices that are suitable for metabolomics studies must fulfill several requirements, such as low chemical background in the mass region of interest (usually 50- 1500 m/z), high ionization efficiency for target compounds, and good co-crystallization (for a series of solvents). Only few matrices exist that fulfill all of these criteria: 9-aminoacridine (9-AA) [1], 1,8-bis (dimethylamino) naphthalene (DMAN) [2], and dihydroxybenzoic acid (DHB) [3]. In this study, we find new MALDI matrices for the detection of low mass compounds in negative mode. We focus on matrices with two- and three-membered heterocycles substituted with 1 to 3 amino groups. Special attention was paid to choosing compounds with a very high (gas-phase) basicity. Their performance in MALDI-MS was screened and compared with known, well-established matrices. Several heteroaromatic amines, such as ethacridine and 4-amino-2-methylquinoline were found to compare favorably with established matrices, such as 9 AA and DMAN in negative-mode MALDI. Moreover, they also allowed straightforward detection of all three classes of tested metabolites (amino acids, nucleotide phosphates, as well as Krebs cycle intermediates), which is not the case for 9 AA and DMAN.

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PTu-094
13:30 – 14:40

Surface plasmon-induced cluster ion formation from Au, Ag, Pt, and Cu irradiated by visible laser

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Keywords:
LDI-MS ; Clusters ; Surface plasmon ; Nanoparticles.

Novel aspects:
There are two desorption/ionization mechanisms in LDI for metal samples, i.e., strong plasmon electric-field induced cluster ion formation and delayed thermal ablation.

Abstract:
Introduction : The laser desorption ionization (LDI) near the threshold region was investigated using visible laser (532nm) for Au, Ag, Pt, and Cu. It was found that for all the metal samples, only the small-size cluster ions were generated but the formation of small-size neutral clusters was negligible. However, nanoparticles with the size of 10s nm were generated due to ablation. This suggests that there are the two desorption processes : (1) the formation of cluster ions due to the surface plasmon excitation (strong near field) at the moment of laser irradiation and (2) the nano-size neutral particles formation at the later stage by the ablation (heat effect) .

Experimental : In this study, we studied the formation mechanisms of neutral and ionic products for metal samples near the threshold region of LDI. The visible laser of 532 nm green Nd : YAG laser with the fluence in the range of 0.4-2.6 mJ/cm^2 was used. The metal targets examined were Au, Ag, Pt, and Cu. The targets were irradiated by laser in helium gas with pressure of about 4600 Pa. The ions formed by the laser irradiation were sampled through an ion sampling orifice into vacuum and analyzed by a time of flight mass spectrometer (JEOL, AccuTOF) . The LDI source was equipped with the dielectric barrier discharge (DBD) ion source. The barrier discharge was turned on and off during the laser irradiation on the metal samples. The mass spectra with DBD on and off were measured.

Results and Discussion : LDI with DBD off, positive and negative metal cluster ions were formed. The cluster ions are composed of less than 10 atoms. The LDI mass spectra were measured with DBD on and off. To our surprise there were no characteristic differences in mass spectra with DBD on or off. If neural clusters were formed, the mass spectra should change with DBD on. Thus, we concluded that LDI does not produce small-size neutral clusters.
After the laser irradiation, SEM images of the metal targets were observed. The surfaces were found to be eroded by laser irradiation and numerous nano particles were found to be formed around the laser spot. These experimental results suggest that the cluster ions are formed by the strong near field at the early stage of laser irradiation, and later on ablation takes place by the temperature rise caused by the relaxation of laser energy.
When 2,4,6-trinitrotoluene (TNT) that was deposited on the laser-ablated metal surface was irradiated by laser, TNT was detected as negative ions, TNT^-. The limit of detection was found to be lower than that obtained for the original metal substrate before laser irradiation. This suggests the plasmon-assisted desorption/ionization for TNT.

PTu-095
11:10 – 12:20

Design and synthesis of new efficient MALDI-MS matrices for low-molecular weight metabolites in negative ionization mode.

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Keywords:
MALDI-MS, matrix, 9-aminoacridine, synthesis, metabolite

Novel aspects:
New efficient matrices, which were designed and synthesized based on 9-AA, for MALDIMS have been developed for low-molecular metabolite analysis in negative ionization mode.

Abstract:
Purpose : Recent years, MALDI-MS has been used not only for biopolymer analysis but also for low-molecular-weight metabolite analysis because it is a highly sensitive, high-throughput, and low sample-consuming technique compared with other conventional analytical platforms. Although metabolites are predominantly organic acids or phosphorylated moieties, making them anionic over a wide pH range, negative ionization mode has rarely been utilized due to poor sensitivity with commonly used matrices. Some of the authors (DM, YF, and HW) have recently reported that the MALDI-MS-system with 9-aminoacridine (9-AA) as a matrix improves the sensitivity of low-molecular weight metabolite analysis in negative ionization mode.[1] However, even by using 9-AA as a matrix, there are numerous low-molecular weight metabolites which cannot be ionized. Although development of more effective matrices has been required, especially for metabolomics. systematic study on the matrix including structure-function relationship has rarely been reported so far. Herein, we report molecular design, synthesis and evaluation of new matrices for low-molecular metabolite MALDIMS analysis in negative mode.
Method : More than 40 compounds including N- or C-substituted 9-AA analogues, anthracene derivatives and quinoline derivatives were synthesized as MALDI-MS matrix candidates. Using these synthetic matrices, 230 kinds of low-molecular weight metabolites were measured by MALDI-TOF-MS in negative ionization mode. The obtained spectra were compared with those obtained using 9-AA as a matrix in terms of sensitivity and S/N.
Results and Discussion: Several new synthetic matrices were found to be more efficient than 9-AA, depending on the metabolites. For example, while the use of 9-AA could detect only 3 of the 40 amino acids and 5 of the 40 carboxylic acids, 9-aminoantracene provided a much better analyte signal, allowing 12 amino acids and 20 carboxylic acids to be detected in negative mode. These results indicated that more potent matrices could be developed with organic synthesis. We will also report our recent results on the structure function relationship of the matrices.

References : 1) *Anal. Chem.***2010**, *87*, 9789 ; *Anal. Chem.***2010**, *82*, 4278 ; *Anal. Chem.***2010**, *82*, 498.
Acknowledgements : This work was partially supported by KAKENHI (24659007, Grants-in-Aid for Challenging Exploratory Research) and the Program for Promotion of Basic and Applied Research for Innovations in the Bio-oriented Industry (BRAIN) .

PTu-096
13:30 – 14:40

Femtosecond Time-Resolved Mass Spectrometry for Desorption Dynamics of Tetracene Ion from Tetracene-doped Anthracene Crystals

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Keywords:
MALDI, Desorption, Femtosecond

Novel aspects:
Desorption dynamics of tetracene ion from teracene-doped anthracene crystals was investigated by means of femtosecond time-resolved mass spectrometry.

Abstract:
Matrix-assisted laser desorption ionization (MALDI) has been widely used as a valuable tool for the analysis in many research fields since operation and preparation of samples are quite easy compared with other soft ionization methods. However, there are several drawbacks at the same time ; 1) low efficiency for ionization, 2) application for low-molecular-weight compounds, 3) not applicable to all kinds of compounds. In order to develop a useful technique for MALDI mass spectrometry, it is also important to fully understand desorption and ionization processes. Much effort has been directed to understand the ionization process by many researchers. Although knowledge of the ionization process in MALDI has accumulated, the mechanism of the desorption process remains unclear. In this study, we developed a femtosecond time-resolved mass spectrometer to probe a desorption process. In conventional MALDI, the roles of matrix and analyte are complicated and not always clear, which sometimes hinder the comprehensive understanding of the MALDI mechanism. To simplify the problem, tetracene-doped anthracene (TDA) crystals, in which efficient energy transfer from anthracene to tetracene occurs, are used as a model system and the desorption dynamics of tetracene ion ([tetracene]$^+$) from the anthracene crystals is investigated [1].
Using a 400 nm pump pulse, anthracene molecules in TDA crystals are initially excited to the S_1 state. After a certain delay (< 10 ps) of pumping, the excitation energy in the S_1 anthracene is transferred to a tetracene molecule to produce the S_1 state. Tetracene in the S_1 state is then ionized by a time-delayed probe pulse (266 nm) and desorbed tetracene ions from the crystals are monitored with a time-of-flight mass spectrometer. The rise time constant of tetracene ion, which is considered to the desorption time from the crystals, was determined to be 94.7 ps for TDA crystals with anthracene : tetracene concentration ratios of 1 : 0.01. The results showed that the desorption of analyte ions was governed by the electronic relaxation of matrix molecules, which could lead to excitation of dissociative modes between matrix and analyte.

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PTu-097
11:10 – 12:20

Matrix-assisted variable wavelength laser desorption ionization of peptides; Influence of the matrix absorption coefficient on expansion cooling

Sung Hee Ahn, Yong Jin Bae, Myung Soo Kim

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Keywords:
expansion cooling, absorption coefficient

Novel aspects:
The temperature drop between early and late plume was measured by using the various wavelengths in matrix-assisted laser desorption ionization. The results support the postulation of the expansion cooling.

Abstract:
The product ion yields in the in- and post-source decays of three model peptide ions, $[Y_5X + H]^+$ (X = Y (tyrosine) , K (lysine) , and R (arginine)) , generated by matrix-assisted laser desorption ionization (MALDI) were measured at six wavelengths, 307, 317, 327, 337, 347, and 357 nm, using α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) as the matrices. The spot size of the laser was kept the same regardless of the wavelength. The laser pulse energy used was two times the threshold value determined for each matrix at each wavelength. The temperatures of the early and late plumes generated by MALDI were estimated via kinetic analysis of the product ion yield data. For both matrices, the temperature drop, i.e. the difference in the temperature between the early and late plumes, displayed negative correlation with the matrix absorption coefficient. This was in agreement with the previous qualitative reasoning that deeper laser penetration and larger amount of material ablation arising from smaller absorption coefficient would result in larger extent of expansion cooling. More importantly, the results support the postulation of the expansion cooling occurring in the plume presented in a previous study.

PTu-098
13:30 – 14:40

Accompanying Compounds induced dehydration of Reserpine in Matrix-assisted Laser Desorption/Ionization Process

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Keywords:
MALDI matrix-effect reserpine dehydrogenation

Novel aspects:
We studied the matrix effects of the dehydrogenation reaction of reserpine. The oxidative dehydrogenation of reserpine in the MALDI process was influenced by functional groups property of matrices.

Abstract:
A plant alkaloid reserpine, extracted from *Rauwolfia serpentina*, is used as a neural drug such as antipsychoticity and antihypertensive. The chemical formula is $C_{33}H_{40}N_2O_9$, and the molecular structure contains two types of N atoms ; one of the indole type (sp^2-hybridized) and the other is a tertiary N (sp^3-hybridized) . It was reported that hydrogen atoms were easily eliminated from the protonated molecule of reserpine $[M + H]^+$ (m/z 608) when the conventional electrospray emitter was used at low solution flow rate. The dehydrogenation molecule of reserpine was observed in the MALDI-MS spectra. In the same manner, those molecules would be generated at the MALDI ionization process. Until now, there have been no studies for the oxidation reaction of low molecule compounds in MALDI process with matrices and/or accompanying components. In this work, we studied the matrix effects of the dehydrogenation reaction of reserpine.
MALDI-MS spectra were obtained using 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (CHCA) , 5-nitrosalicylic acid (NSA) , 1,5-diaminonaphthalene (DAN) , 5-aminosalicylic acid (ASA) , 5-formylsalicylic acid (FSA) as a matrix. Yohinbin, 1,2,3,4-tetrahydro-9H-pyrido [3,4-b] indole and norharman were used for revealed a dehydrogenation part.
At first, whether matrix molecules influences dehydrogenation of reserpine. MALDI-MS spectra of reserpine (m/z 608) was observed protonation ion $[M + H]^+$ as a most abundant using hydrogen donating ability group (-OH or NH_2) matrices such as DHB and DAN, ASA, and the dehydrogenation ion $[M - 2H + H]^+$ abundance was low. On the other hands, dehydrogenation ion $[M - 2H + H]^+$ as a most abundant ion observed using hydrogen acceptability matrix such as NSA and FSA. Therefore, the oxidative dehydrogenation of reserpine in the MALDI process was influenced by functional groups property of matrix.
Following, yohinbin, 1,2,3,4-tetrahydro-9H-pyrido [3,4-b] indole and norharman with a partial structure of reserpine were analyzed to examine a dehydrogenation part of reserpine in MALDI. The dehydrogenation ion was not detected in only 1,2,3,4-tetrahydro-9H-pyrido [3,4-b] indole without tertiary N atom. Consequently, it was revealed that the dehydrogenation process was caused by a proton adduct to tertiary amine.

PTu-100
13:30 – 14:40

Highly-Soft-Ionization of Organic Compounds and Polymers by Using Semiconductor Nanoparticels Sample Plate

Tetsu Yonezawa[1], Hayashi Shinji[2], Suganuma Takashi[3], Hideya Kawasaki[3], Ryuichi Arakawa[3]

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Keywords:
GaP, SALDI-MS, Soft Ionization, Particle, Color

Novel aspects:
Usually SALDI-MS shows lower soft ionization ability than MALDI-MS but GaP nanoparticles overcome this problem.

Abstract:
MALDI-TOF-MS with organic matrices is a common soft ionization technique that features minimal fragmentation of mass analysis especially for bio-molecules and polymers. Various UV-absorbing organic acids including 2,5-dihydroxybenzoic acid (DHBA) and a-cyano- 4 -hydroxycinnamic acid (CHCA) were introduced into MALDI system, and used to analyze various compounds. However, MALDI-MS has some weak points, the most severe one is fragment peaks corresponding to the matrix compounds which are observed in the mass region less than 500 Da. In this mass range, however, one can find many medicals and toxic compounds. In order to overcome this problem, various organic-matrix-free systems have been introduced to realize the mass analyses of small molecules. Among various systems, including DIOS and others, nanoparticle (NP) -based SALDI-MS has been proposed increasingly during the last decade. Various inorganic NPs including metal oxides, metals, semiconductors, have been applied for SALDI-MS inorganic matrices.
We have investigated the feasibility of GaP nanoparticles for SALDI-MS. GaP is one of the important semiconductor materials, especially for light emitting diode (LED) . GaP NPs were prepared gas-evaporation method and show yellow dark red color according to their sizes. These NPs were directly deposited onto stainless steel sample plates without addition of any stabilizing reagent. Yellow particles are smaller and red particles are larger. This is attributed to the bandgap of GaP NPs. We tried to obtain a mass spectrum of polymers (PEG) with these GaP NP substrate. The fragmentation behavior strongly depended on the particle color. Red particles showed no fragmentation but yellow ones showed many fragment peaks at lower mass region. Survival yield measurements also strongly supported these data, which indicated that SALDI-MS with GaP nanoparticles show highly soft ionization of the organic compounds and the obtained survival yield is same as MALDI-MS systems with organic matrix molecules.

PTu-101
11:10 – 12:20

Desorption/Ionization efficiency of 20 common amino acids in SALDI-MS using platinum nanoflowers on silicon substrate

Shuhei Nitta, Hideya Kawasaki, Ryuichi Arakawa

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Keywords:
SALDI-MS, amino acids, proton affinity, acidity

Novel aspects:
We found that the Pt Nf-SALDI efficiency strongly depends on the kinds of 20 common amino acids as well as the ion modes (i.e. negative or positive) .

Abstract:
Matrix assisted laser desorption/ionization (MALDI) is known as one of the soft ionizing methods of mass spectrometry (MS) .[1] However, it is sometimes difficult to analyze small molecule-samples because of the presence of interference-ion peaks from organic matrixes in the MALDI mass spectra. In contrast, surface assisted laser desorption/ionization (SALDI-MS) using inorganic matrixes such metal nanoparticles or nanostructured has some advantages on the detection of small molecule-samples because of low interference-ion peaks from inorganic matrixes.[2] Recently, we have reported that the SALDI-MS by using a platinum nanoflower (Pt Nf) with nano-scale sharp edges.[3] Although the desorption/ionization efficiency strongly depends on the chemical structures of analytes, the relation between the chemical structure of a sample and the SALDI efficiency is not clear. Thus, we have examined the relation between the chemical structure of analytes and the SALDI efficiency by using model analytes of 20 common amino acids with various side chains. We found that the Pt Nf-SALDI efficiency strongly depends on the kinds of amino acids as well as the ion modes (i.e. negative or positive) .
We obtained the relative ion peak intensities (I_r) of 20 common amino acids to that of the angiotensin II as an internal standard. All amino acids can be detected as a proton adduct form. The value of I_r was largest for arginine in positive ion mode, while that of Ir was the largest for aspartic acid in negative ion mode. There was a good positive correlation between the proton affinity (PA) of amino acids in gas phases and the I_r in positive ion mode. In negative ion mode, we obtained the correlation between the gaseous phase acidity (GA) an of amino acids in gas phases and the I_r. These results suggest that the desorption/ionization process on Pt Nf-SALDI are similar to those of MALDI-MS using organic matrixes. It should be noted that the dominant detection of protonated molecular ions in the Pt Nf-SALDI much differs from those of other SALDI-MS, where the dominant detection of molecular ions was metal-adduct ions (e.g. sodium adduct ions) .[3] The high efficiency of protonaed ions might be attributed to the acidity-increases of OH groups on the silicon by the deposition of Pt nanoparticles. [4]

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PTu-102
13:30 – 14:40

Hydrogen Removing and Compound Properties of Aromatic Carboxylic Acids for Negative-ion Generation in Matrix-assisted Laser Desorption/ Ionization Mass Spectrometry

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Keywords:
MALDI, isotope pattern, DHBA

Novel aspects:
Only 2,5-dihydroxy benzoic acid (DHBA) showed a special property highly generating a hydrogen radical among six DHBA isomers.

Abstract:
Analysis of isotope patterns in MS signals are powerful methods for elucidation of chemical formula of analytes. Isotope patterns are simulated and those are compared with experimental data. Generally, the calculation was performed for one chemical species. It was important for us to determine the chemical species contributing the isotope patterns of the MS signals if a few chemical species overlapped. In negative-ion matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) , aromatic carboxylic acids have aromatic parts producing a molecular ion $[M]^-$ and a negative-charged carboxylic acid part generating a deprotonated molecule $[M - H]^-$. The observed MS signals were generated both from molecular ion $[M]^-$ and deprotonatd molecule $[M - H]^-$. They differed 1 Da, and overlapped each other. These complex overlapped isotope patterns could hinder elucidation of the chemical formula of the analyte if it was unknown compounds. Moreover, hydrogen removing from deprotonated molecules was observed in the negative-ion mode MALDI-MS of aromatic compounds having phenolitic hydroxyl groups, which caused the more complex isotope patterns from more than three different chemical species. Here, we studied ionization tendency of aromatic carboxylic acids and those having hydroxyl groups. It is necessary to translate complex isotope patterns of aromatic carboxylic acids having hydroxyl groups because those structural properties are in many natural compounds.
In the actual experimental data, benzoic acid was ionized as a deprotonated molecule $[M - H]^-$. In contrast, both of the molecular ion $[M]^-$ and deprotonated molecule $[M - H]^-$ were generated from 1-naphtoic acid, and the ratio of molecular ion to deprotonated molecule $[M]^-$ / $[M - H]^-$ was increased in 1-anthoracenic acid. Aromaticity of the compounds tends to lead generation of molecular ion $[M]^-$. In addition to that, the ratios differed among three anthracitic acid isomers, which made it difficult to predict the isotope patterns. 2,5-Dihydroxy benzoic acid (2,5-DHBA) showed two deprotonated molecules such as $[M - H]^-$ and $[M - H - H]^-$ which was generated from a hydrogen removing from a phenolictic hydroxyl group. The generation of the third chemical species hindered translation of the isotope patterns, identification of the compounds, and elucidation of the chemical formula. Only 2,5-DHBA showed the special property highly generating a hydrogen radical among six DHBA isomers.

PTu-103
11:10 – 12:20

Matrix-assisted Laser Desorption Ionization Mass Spectrometry Using Cation-Substituted Zeolite Surface

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Keywords:
MALDI, Zeolite, Alkali metal cation

Novel aspects:
An efficient matrix which is applicable to the compounds that cannot be detected by conventional MALDI was developed by using 2,4,6-trihydroxyacetophenone (THAP) adsorbed on cation-substituted zeolite surface.

Abstract:
Matrix-assisted laser desorption ionization (MALDI) in combination with time-of-flight (TOF) mass spectrometry (MALDI-MS) has been widely used in many research fields because it enables observation of analyte ions in terms of their molecular weights. MALDI-MS offers other advantages as well, including tolerance to contamination, a mass spectrum consisting of mostly singly charged ions, and simple instrumentation. Of course, MALDI also has drawbacks. In MALDI-MS, usually, a peak of a protonated analyte, $[M+H]^+$, is observed ; however, the ion yield of the protonated analyte is sometimes influenced by the presence of alkali metal ions. As biological samples contain an abundance of alkali metal ions intrinsically, the peak intensity of the protonated analyte is suppressed by peaks of alkali metal ion adducts. Secondly, MALDI is not suited for the study of low-molecular-weight compounds. In MALDI-MS measurements, an analyte is mixed with a large amount of matrix molecules. The analyte undergoes soft ionization for which it does not dissociate ; however, the matrix molecules that absorb photons from an excitation laser dissociate, producing many matrix-related peaks in the low-molecular-weight region less than 500 Da as the molecular weight of a typical MALDI matrix is less than 500 Da. Thirdly, the mechanisms of desorption and ionization are still unclear. Because of lack of fundamental knowledge of the desorption and ionization mechanisms, the choice of matrix molecule suited for a particular study is done by trial and error. This hinders the detection of many kinds of molecules by this method. To solve these problems, many attempts have been made to use various compounds as co-matrices, and those attempts have produced good results. A matrix-free technique, such as DIOS and the use of a nano-structured surface have been proposed as well. Recently, we used cyclodextrin and zeolite as host molecules for typical MALDI matrices and found that they suppressed the fragmentation of a guest matrix molecule and increased the peak intensities of the protonated analytes.
In this study, we also used zeolite. Zeolites are crystalline aluminosilicates with nanometer order cages, and usually act as solid acid catalysts for such processes as cracking, isomerization, and alkylation of hydrocarbons. Zeolites have high catalytic acidity due to the charge imbalance at the Si-O-Al bridging sites, and those sites are compensated by such cations as H^+ and Na^+. In particular, hydroxyl (OH) groups having Brönsted acidity exist in H^+-exchanged zeolite, and it is well known that the Brönsted acidity is responsible for various catalytic activities. In this study, we exchanged proton on the zeolite surface with alkali metal cations. By using 2,4,6-trihydroxyacetophenone (THAP) adsorbed on cation-substituted zeolite as the new matrix (zeolite matrix) , we succeeded in observing large ion peaks of cation-adducted analytes (low-molecular-weight compounds) . It was also found that the zeolite matrix is applicable to compounds such as acethylsalicylic acid, barbital, phenobarbital, colchicine, digoxin, amygdalin, and so on, which cannot be detected by conventional MALDI. We are fully convinced that the zeolite matrix can further improve the applicability of MALDI-MS.

PTu-104
13:30 – 14:40

Correlation of Ionized Carbohydrate Structure with Peak Intensity of Negative Ion Mass Spectrometry: First Principles Study

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Keywords:
MALDI, Negative ion CID, Carbohydrate, quantum calculation, ionization energy

Novel aspects:
This study would be the first study that investigated the stability of deprotonated structures and the correlation of mass intensity with its ionization energy for longer oligosaccharides by quantum calculation.

Abstract:
Matrix-assisted laser desorption/ionization multiple-stage mass spectrometry (MS^n) has been essential techniques in characterization of carbohydrates. In particular, negative ion collision induced dissociation (CID) spectra provides detailed structural information of oligosaccharides by specific fragmentation patterns. Fragmentation is assumed to occur firstly from deprotonation of certain hydroxyl group of a carbohydrate, and then bond cleavages occur according to the patterns of electron transfer from a deprotonated site to other sites. Original structure could be deduced according to the fragmentation patterns. This fact means that deprotonation process is of fundamental for MS^n spectrum acquisition because fragmentation patterns are mainly specified by a location of the deprotonation site. Considering deprotonation process at a hydroxyl group, there are many possible sites for the deprotonation in the carbohydrate. Ordering of frequency of deprotonation would depend on the stability of the deprotonated structure varying by the interaction between the ionized oxygen atom and the other atoms in the molecule via the hydrogen bonding interaction or by the delocalization effect of electron. The degree of stability of a deprotonation site will show the correlation with the signal intensity because energetically stable fragment is favorable to forming in CID which resulting in a high intensity of the signal. Therefore, understanding of the stability of deprotonated structure of oligosaccharide is important for the better understanding of fragmentation. However, as long as our knowledge, the nature of deprotonation process of longer oligosaccharides has not been studied.
In this study, atomistic structure and energetically stability of deprotonated structures for several oligosaccharides, e.g. NA2, MFLNH and maltopentaose, were investigated by first principles calculations. Then the energy of deprotonated structures was compared with our experimentally obtained MS^ndata and correlated with the intensity of the spectrum. We found a good relationship between the stability of deprotonated structure and the intensity of the spectrum. The origin of stability of the deprotonated structure was also investigated in the term of the specific interaction such as hydrogen bonding. The results show the existence of the rule of stability for deprotonated sites. The rule will be used for the theoretical prediction and better understanding of MS^n spectrum. In the presentation the details of results and theoretical method for the prediction of intensity of spectrum will be presented.

Acknowledgement
This work was supported by JST, Japan Science and Technology Agency (Development of Systems and Technology for Advanced Measurement and Analysis) .

PTu-105
11:10 – 12:20

Statistical Investigation of Metabolite Structure to Reveal the Principle for Preferred Ionization in MALDI using 9-Aminoacridine

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Keywords:
MALDI-MS,metabolite analysis,9-aminoacridine,molecular descriptor,ionization

Novel aspects:
The ionizability of metabolites in 9-AA-MALDI was characterized using molecular descriptors.

Abstract:

Background

9-Aminoacridine (9-AA) is one of the matrices most often used for low-molecular-weight metabolite analysis by negative ion mode matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). To date, 9-AA has been proven to be capable of ionizing a series of metabolites such as phosphorylated compounds, and utilized for various studies including sensitive detection of metabolites and metabolite imaging. However, to clarify whether a metabolite is detectable, an experimental trial is needed. As for metabolites that have not been detected by 9-AA, we may need to employ drastic strategy such as rational alteration of matrix to cover them. Although the ionizability of analyte in MALDI decisively depends on matrices, it is still elusive which factor dominates the efficiency of the ionization. In this study, we investigate the interdependency of the chemical structures of metabolites and their ionization efficiency assisted by 9-AA in MALDI-MS. Quantitative structure-property relationship (QSPR) was applied to associate the structure of metabolites with their ionization efficiency in MALDI. A merit of property modeling is not only a rapid property estimation of unexamined compounds but also useful information about the structure features that are relevant to desired properties.

Results

MALDI-MS analysis of metabolite standards. The ionization capability of 9-AA was evaluated using 233 standard compounds of metabolites selected to general importance. Individual standard compound was diluted to graded concentrations and mixed with 9-AA/MeOH solution (10 mg/mL) by 1 : 1 (v/v). The mixture was then subjected to MALDI-TOF-MS analysis to confirm the possibility of ionization and the limit of detection (LOD). As the result, 121 out of 233 metabolites were detected as deprotonated peaks. LOD varied from 10 fmol/well to 100~ μmol/well. We used the LOD value as an inverse measure of the ionization efficiency at best on an ordinal scale.

QSPR analysis. A QSPR analysis is constituted of five steps : MALDI-MS data collection, descriptor calculation, variable selection, predictive model construction through cross validation, and interpretation of the model. The molecular descriptors and fingerprints were calculated by PaDEL-Descriptor[1] using MDL-molfile formatted files of individual metabolites acquired from KEGG (http : //www.genome.jp/kegg/) or HMDB (http : //www.hmdb.ca/). Modeling of interrelationship between the descriptors and ionization efficiency of metabolites under 9-AA-MALDI was conducted using several statistic method including logistic regression, generalized linear model (GLM) and Random Forests. All the statistical analyses were performed using R (http : //www.r-project.org/). First we explored suitable combination of descriptors to predict the ionization capability, expressed as a response variable categorized as 'ionized' or 'not ionized' ; or 'highly effective' or 'less effective', and so on, regarding the value of LOD. Random Forests was employed for classification to select relevant variables. At this point, atom type electrotopological state indices were found potentially relevant to ionization capability, and autocorrelation descriptor to the efficiency. As these descriptors are indices reflecting molecular connectivity and constituting elements, potential rule for preferable ionization may be extracted. In the session, construction of regression models will be discussed.

Conclusion

This study was primarily intended to lead 9-AA-MALDI-MS analysis to be placed on a rational and predictive foundation. Relevant descriptors found in this study can be interpreted as the structural preference specific to 9-AA. Upcoming question is that, when the structure of matrix alters, how the preference shall shift. This information will play an indispensable role on strategic control of MALDI-MS-based studies.

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PTu-106
13:30 – 14:40

Matrix clusters distribution from monomer to clusters with the mass at mega Dalton's region

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Keywords:
MALDI, Matrix Clusters, Sinapic acid

Novel aspects:
We gave the first demonstration for quantitative analysis of matrix clusters distribution from monomer to clusters with mass-to-charge (M/z) ratio reaching 2,000,000.

Abstract:
Introduction
Many publications have shown that matrix clusters play an important role in MALDI process. In this work, we try to demonstrate a completely matrix clusters distribution from monomer to clusters with the mass at mega Dalton's region by MALDI-TOF and our homemade MALDI- quadrupole ion trap mass spectrometer. This frequency scanned ion-trap system can quantitatively detect clusters signals by charge detector for high mass-to-charge ratio (m/z) from 20,000 to 2,000,000. To our knowledge, this is the first presentation for matrix clusters distributing to super-heavy region.

Methods
Compare to commercial quadrupole ion trap, lower frequency (10 kHz - 50 kHz) was used for our homemade QIT to trap ions with large m/z. In order to quantitate the clusters amount, we fix the q_z value scanned from 0.908 to 0.5 keeping the same condition of potential well. Trapping frequency can be changed to cover the different m/z regions which can be calculated by $q_z$value and RF voltage. In general, a broadband power amplier (TREK, PZD2000A) was used to sustain RF voltage between 10 kHz to 50 kHz and frequency scan was used to identify the m/z values of the ions.

Preliminary Data
We sketch the sinapic acid (SA) clusters distribution from monomer to large clusters with m/z reaching 2,000,000. The signal intensity drops about 6 - 7 orders of magnitude from monomer to 5000 mers.

PTu-107
11:10 – 12:20

The initial ion velocity of MALDI-TOFMS matrix DCTB and affect upon the mass accuracy of high molecular weight radical ions.

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Keywords:
MALDI ; DCTB ; ion velocity ; radical ions ; calibration

Novel aspects:
Previously undetermined initial DCTB matrix ion velocity and improved calibration of higher molecular weight radical ions.

Abstract:
One of the early investigations into the fundamentals of the matrix-assisted laser desorption/ionisation (MALDI) mechanism revealed that the initial ion velocity of the matrix varied between matrices, and that this velocity affected the qualitative calibration or mass accuracy of the analyte being characterised. The initial ion velocities of all the common MALDI matrices were measured and incorporated into the calibration software of commercial MALDI-TOFMS instruments, so the analyst would choose the matrix they were using in the software and so obtain the best possible mass accuracy. However, the applications of MALDI have continued to increase and with that have come several new matrices available to the analyst. One of these, 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]-malononitrile (DCTB), has proved very useful for characterising aryl, aromatic and metal-containing analytes via the electron-transfer mechanism to produce radical ions. The initial ion velocities of these new matrices were often not measured, so the analyst would use the median of known values or try all values with a known analyte before selecting the best fit for future use. When using the initial ion velocity of another matrix with DCTB analyses, the observed affect upon mass accuracy may be insignificant for small molecules, but significantly different for mass values of high mass analytes.
For a range of known medium to high mass analytes, the initial ion velocity of DCTB has been estimated by successive analyses using known values and comparing the observed mass accuracy. The initial ion velocity of DCTB has also been determined directly, and the data from both approaches is compared and discussed in terms of the affect upon mass accuracy, and the wider aspects of the MALDI mechanism.

PTu-108
13:30 – 14:40

Gas-Phase Fragmentation of Reducing Sugar Modified Small Oligosaccharides

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Keywords:
oligosaccharides, modification, ion fragmentation chemistry

Novel aspects:
Detail study of the fragmentation chemistry (the charge location effect and the sidedness of the fragmentation) using different types of modifications on reducing end of oligosaccharides.

Abstract:
Carbohydrates play important roles in essentially all living organisms. Understanding these roles requires that their structures be determined with confidence. This involves all levels of structural characterization, including the stereochemistry of each sugar, the anomeric configurations, the linkage positions between connecting sugars, and any modifications of sugars. Tandem mass spectrometry (MS^n) has been widely applied in structural analysis of carbohydrates for determining the sequence, branching, linkage type, and positions of substituents. Collision-induced dissociation (CID) spectra of carbohydrates typically show very complicated fragmentations due to the existence of structural isomers. Detail understanding of the fragmentation mechanisms is important for the characterization of carbohydrates.

Four types of modifications, such as ^{18}O, 4-aminobenzoic acid (ABA), 3-amino-2-napthoic acid (ANA), and ethyl-*p*-aminobenzoate (ABEE) were used on disaccharides to pentasaccharides. ^{18}O-labeling at the carbonyl oxygen of the reducing sugar allows the distinction of the sidedness of the fragmentation pathway and mass discrimination of structural isomers. Sixteen different disaccharides which are the smallest substructures derived from oligosaccharides that still contain a glycosidic linkage between two monosaccharides were systematically studied. Comparisons of the MS^2 CID spectra between ^{18}O-labeled and non-labeled disaccharides showed that there were some doublet ion peaks (offset of 2 m/z) indicating the existence of the structural isomers with or without ^{18}O. These ions were further examined by MS^3 CID. The *m/z* 163 ion was a highly abundant ion that was observed beside *m/z* 161 ion in MS^2 spectra of all ^{18}O-labeled disaccharides and has the possible structure of anhydromonosaccharide (reducing sugar) containing ^{18}O. Further CID of this ion showed a distinct CID fragmentation patterns according only to their linkage positions but did not show any differences between anomeric configurations and stereochemistry of the sugar. ABA, ANA, and ABEE modifications were used to study the effect of charge location. The fragmentation chemistry of the modified disaccharides and trissacharides will be studied.

PTu-109
11:10 – 12:20

Radiosensitizers investigated using electrospray ionization mass spectrometry

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Keywords:
nimorazole, misonidazole, radiosensitizer, hypoxia, electrospray ionization

Novel aspects:
First investigation of radiosensitizer compounds using mass spectrometry. A key finding is that radical anions M.- are directly formed via electrospray ionization.

Abstract:
Radiosensitizers are used in radiotherapy (following the DAHANCA 5 clinical trial) to enhance tumour control of radioresistant hypoxic tumours. Radiosensitizers implemented particularly in the treatment of hypoxic cells are called 'electron-affinic' radiosensitizers. A number of nitroimidazoles and related compounds were studied in vitro as well as in vivo, with encouraging results. However, the detailed mechanism of the actual radiosensitization is still unknown. A working hypothesis is that these compounds undergo redox reactions inside the cell, and that the nitroimidazole rings facilitate reduction via the formation of radical anions.

The work presented here investigates the formation of nimorazole and misonidazole ions via positive and negative electrospray ionization mass spectrometry, and the subsequent fragmentation reactions of the cations and anions. Understanding the ionization and fragmentation of radiosensitizers is crucial in evaluating the radiosensitization potential and developing new and more effective drugs, which may improve tumour control probability in hypoxic tumours when using ion beams.

The experiments have been performed using a Finnigan- LTQ-FT mass spectrometer equipped with an electrospray ionisation source. Collision-induced dissociation (CID) and electron-induced dissociation (EID) have been carried out by mass selecting the desired ions and subjecting them to activation energy in the linear trap and to free electrons of 25.8 eV in the FT-ICR cell. We have examined CID and EID of protonated nimorazole and protonated misonidazole and CID of nimorazole and misonidazole radical anion.

Electrospray ionisation of nimorazole in the positive ion mode yields protonated nimorazole $[M+H]^+$, with no radical cation formation being observed. The major fragment ion in the CID spectrum of protonated nimorazole occurs at m/z 114 and arises from loss of the nitroimidazole ring. Although this fragment is also observed in the EID spectrum, the dominant fragment in the EID spectrum is m/z 110, due to the loss of nitroimidazole ring and CH_2 group. In both spectra, a minor loss of NO_2 radical is present. In negative ion electrospray ionization, a radical anion of nimorazole $M^{\cdot-}$ can be formed. CID of $M^{\cdot-}$ generates amongst others the negatively charged nitroimidazole ring at m/z 112 and a peak corresponding to a minor loss of NO radical.

In the case of misonidazole, the CID spectrum of protonated misonidazole shows the major fragment to be also an ion at m/z 114, but in this case it is the nitroimidazole ring, which holds the charge after CID. This fragment is also the most dominant fragment in the EID of the protonated misonidazole $[M+H]^+$.In contrast to CID of the protonated nimorazole, all the CID fragments of protonated misonidazole $[M+H]^+$ correspond to fragments, where the positive charge is retained by nitroimidazole ring. Only one fragment of m/z 89, where the charge is located on the side chain, was observed. Negative ion electrospray ionization of misonidazole also readily forms the radical anion $M^{\cdot-}$.The CID of the radical anion of misonidazole shows much more fragmentation than in the case of nimorazole. Most of them include the loss of NO and NO_2 groups. The negatively charged nitroimidazole ring at m/z 112 is also observed as in the case of nimorazole.

The fragmentation of both radiosensitizers appears to depend on the nature of the charge. This detailed gas-phase study will unlock their fragmentation mechanism and highlight the similarities and differences in the fragmentation products observed.

PTu-110
13:30 – 14:40

Expected possibilities of a new type gas-dynamic interface for preliminary separation and transportation of ions into a mass analyzer

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Keywords:
supersonic flow ; EI ; RFQ ; ortho-TOFMS

Novel aspects:
Supersonic gas flow formation inside a capillary is confirmed experimentally. The estimated temperature of Ar flow is below 0.01K. Two-grid mirror is proposed to extract ions from the flow.

Abstract:
A basic problem of mass spectrometric analysis of samples initially being under atmospheric pressure is an efficient input of either or both an analyzed gas mixture and ionized species into a vacuum part of the instrument. We have described recently one possible way to solve this problem [1, 2] . Its essence is using relatively long thin capillary at rather low initial pressure of a gas to form a supersonic gas stream. This stream goes through a high-performance ion source with ionization by electrons with controlled energy and further along the axis of the radio-frequency quadrupole (RFQ) coupled with time-of-flight mass analyzer with orthogonal injection of ions (ortho-TOFMS) .

Relatively large gas density in the flow along RFQ axis and fast decreasing this density outside the axial zone are specific features of the considered case in comparison to conventional gas-filled RFQ. They provide additional opportunities to accumulate and preliminary separate the target ions and simultaneously to eliminate preventing ion flows of basic components of investigated mixture unimportant for analysis. The experimental data of cross-section distributions of recorded Xe ion flow from argon - xenon gas mixture clearly show ring-like ion distribution around stronger focused beam of argon ions along the RFQ axis. Applying appropriate axial electric field to stop ions inside RFQ, such ion stratification may be used for selective accumulation and for collision induced dissociation of target ions. Similar and additional possibilities for ion manipulation may be implemented in case of absence of the buffer gas ions in RFQ. This situation may be realized by reducing the electron ionization energy or by increasing the RF focusing voltage, which results in instability of motion of small ions.

Experimental data analysis shows that only very small share of the ions formed inside supersonic stream is recorded by existing system. Rather high density of this stream at the quadrupole exit does not allow to extract the most part of the ions from the stream and to give them energy sufficient for recording by the ortho-TOFMS. To eliminate these difficulties, one can move the ions from the gas stream orthogonally to its axis by two-grid electrostatic mirror and to couple the ortho-TOFMS orthogonally to the existing gas-dynamic interface. The carried out computer simulation has shown efficiency of such approach with additional possibility to separate ions according to their mechanic energy in the vicinity of the mirror. Hopefully, realization of supposed orthogonal coupling will provide high level of sensitivity and selectivity for different kind of analyses, including determination of trace impurities in air without preliminary sample preparation. The work is carried out in the framework of the Program 8 for Basic researches of Presidium of Russian Academy of Science.

1. V.V.Raznikov, V.V.Zelenov New Way to Build a High-Performance Gas-Dynamic Interface to Produce and Transport Ions into a Mass Analyzer. Int.J.Mass Spectrom. (in press) 2012.
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PTu-111
11:10 – 12:20

Dramatically Improved Hydrocarbons Analysis with the 5975-SMB GC-MS with Cold EI

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Tel Aviv University, Tel Aviv, Israel

Keywords:
GC-MS, Cold-EI, Wax, Hydrocarbons, Fuel-Oil

Novel aspects:
GC-MS with supersonic molecular beam and cold-EI of vibrationally-cool molecules in fly-through ion source. Improved hydrocarbons identification and characterization with compounds extended range, enhanced molecular ion, novel isomer abundance analysis.

Abstract:
Hydrocarbon analysis, including fuels, oils and waxes are of vital importance to broad range of applications. However their analysis by GC-MS is confronted with limitations in the elution of large and low volatility compounds and in the absence of trustworthy molecular ions and representative high mass fragments which consequently often precludes proper identification. In addition, such analysis is further exacerbated by the lack of proper LC and/or LC-MS alternatives.
GC-MS with supersonic molecular beams (SMB) and its Cold Electron Ionization (Cold EI) allow to overcome the challenges of hydrocarbon analysis occurring in standard GC-MS technique. Cold EI with it fly-through ion source eliminates the well-known conflict between ion source related peak tailing and molecular ion abundance which originates from the conventional ion source layout as will be explained and discussed. In addition, the SMB based approach significantly increases the range of compounds amenable for GC-MS analysis via lowering the compounds elution temperatures as will be demonstrated.
We used an Aviv Analytical model 5975-SMB GC-MS with Supersonic Molecular Beams (SMB) for hydrocarbons analysis. It is based on the combination of the SMB technology with the Agilent 5975 GC-MS, forming a new and powerful GC-MS system. The GC eluting sample compounds are mixed with helium make up gas in a temperature programmable transfer line, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, and pass a fly-through, electric field free EI ion source where they are ionized by 70 eV electrons as vibrationally cold molecules (hence the name Cold EI) . Ions originated from the beam molecules continue their straight flight, exit the ion source, are reflected at right angle towards the quadrupole MS, are mass analyzed and detected.
GC-MS with SMB enables the analysis of large wax compounds up to C74 via the use of short columns with high column flow rates to ensure their proper elution from the GC, as well as due to the features of fly-through ion source that does not cause any peak tailing of low volatility compounds. GC-MS with Cold EI (using SMB) provides trustworthy and largely enhanced molecular ions to all analytes as well as enhanced high mass fragments, for their improved identification. Isomer abundance analysis is enabled for obtaining unique and powerful hydrocarbon mixtures characterization method, that can be used for geochemical information, fuel and oil characterization and source location in oil spill and arson investigations.
The analysis of the following samples will be presented and described : polywax, oxygenates in wax, flesh flies wax, flower wax, beeswax on fruits and vegetables, jet engine oil, Jojoba oil, Nonoxynol-9 condom spermicide oil, motor oil freezing properties, triglycerides in biodiesel Diesel, fuel characterization, biodiesel in jet fuel and more.

PTu-112
13:30 – 14:40

Suitability of dual solid probe FT-ICR-MS system for gas-phase acidity and basicity measurements of low volatile compounds.

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Keywords:
gas-phase acidity, gas-phase basicity

Novel aspects:
dual solid probe accessory for introduction of compounds for gas-phase acidity and basicity measurements

Abstract:
FT-ICR-MS is one of the most used methods for determination of gas-phase acid-base properties of compounds. Although there is available large data collection of gas-phase acid-base properties[1] there is made only small progress during last decade in the field of experimental research of superacidic and superbasic compounds.[2] At the same time were designed and by using computational chemistry tools were shown that considerably stronger superacids and -bases may exist.[3] Several of these compounds have been synthesized and their strength in solution have been verified, but their gas-phase acid-base properties have not been yet experimentally determined. The main reason is that many of the neutral organic superacids and superbases have high molar masses and very low volatility. Such compounds are difficult to study experimentally as for relative gas-phase acidity or basicity measurements volatile reference compounds are needed on common FT-ICR-MS instruments (single heatable solid probe and several leak valves) , which have been used for that task.
To overcome these experimental difficulties was designed and built together with Varian (later acquired by Agilent) FT-ICR-MS team a dual solid probe inlet accessory and fitted it with Varian 930 MS 7T system. Optimal experimental parameters were determined for the customized system by using previously studied compounds and performance was tested with some low volatile compounds.

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J.Phys. Chem. A,2009, *113*, 12972-12978 ; *J. Am. Chem. Soc.*, 2005, *127*, 17656-17666.

PTu-113
11:10 – 12:20

Development of mass spectrometer equipped with ECRIS and its application to isotopic analysis of Mo

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Keywords:
ECR-IS, molybdenum, isotope, ion sputtering

Novel aspects:
Our results suggest that ECRIS-MS has great potentiality and that it can be widely applied to isotopic analyses with high sensitivity.

Abstract:
[Introduction] The precise measurement of isotope ratios in geological and planetary materials has been expected to give us significant information on their origin. For example, several elements in meteorites, such as Mo and Sn, have been recognized as important for examining the process of nucleosynthesis. Therefore, a development of the mass spectrometer with the potential ion source has been regarded as one of essential subjects in mass spectrometry. We focused our attention on the high potentiality of an electron cyclotron resonance ion source (ECRIS) as an ion source for mass spectrometers and attempted to customize the mass spectrometer equipped with an ECRIS for application to the analysis of Mo isotopes. The ionization efficiency of the ECRIS is generally high, and all elements can be fundamentally ionized. In this study, for metal samples, we employed an ion sputtering system as a sample introduction system.
[Method] The ECRIS consists of the following three parts : permanent magnets for the magnetic mirror field, a high-frequency power supply (10 GHz) , and a plasma chamber. This ion source was combined with ananalyzing magnet followed by a detection system (Faraday cup) . Using the above system, we performed the isotopic analysis of Mo assisted by the sputtering method. To establish ECR plasma, we used Kr or O gas. The sample was sputtered with Kr or O ions produced in the ECR plasma with a potential between 1 and 5 kV. As a sample, we examined several metal materials, such as the SUS 316 standard material, to simulate the analyses of iron meteorites.
[Preliminary results] We confirmed with a mass spectrometer that the sputtering yields of ions obtained using the Kr and O gases are sufficient. Furthermore, the observed signal intensity was very stable in each measurement and the signal intensity changed by less than 0.5% in 1 hour. The observed isotope ratios of Mo in SUS 316 were in good agreement with the data calculated from natural isotope abundances. However, in the case of sputtering with Kr, the precision of isotope ratios decreased owing to the effect of the background noise derived from Kr isotopes (*m/z* 80-86) and several interference peaks observed from *m/z* 100 to 110. The mass spectrum pattern of those interference peaks exhibited the isotope abundance of Kr. Therefore, those peaks are assumed to be derived from a certain cluster of Kr or a similar cluster. To date, the origins of those interference peaks have not been elucidated yet. On the other hand, using O gas for the ECR plasma and sputtering source, a fine background condition was obtained. However, small interference peaks were observed at *m/z* 100 and 101. Those peaks did not depend on the sputtering potential and total ion current. This suggests that those peaks are not derived from the sample but from the circumstance of ion source. It should be necessary to refine the parts of the ion source to decrease those interference peaks. The results of isotope ratios except ^{100}Mo exhibited a high precision within 0.5% and also accordant ratios with natural isotope abundances. Our results suggest that ECRIS-MS can be widely applied to isotopic analyses with high sensitivity.

PTu-114
13:30 – 14:40

Analysis of ion cloud dynamics in diffrent geometry orbitrap mass-analyzers using Particle-in-Cell code based computer simulations

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Keywords:
FTMS, orbitrap, simulation, space charge

Novel aspects:
Deeper understanding of ion clouds behavior in orbitraps of different geometry obtained by supercomputer simulations

Abstract:
Fourier transform mass analyzers based on electrostatic traps technique are attracting the growing interest for their compactness and high performances. The well known orbitrap is a member of more general class of traps - cassinian traps [1], in which it is possible to make ion motion harmonic and measure ion masses via frequencies of oscillation in harmonic potentials. There is great interest to comparative analysis of different geometry variants cassinian traps. In this work we consider the simplest cassinian trap - orbitraps of different geometry. Analytical performance (resolution, dynamic range) of such mass analyzers in general is determined by trap geometry as well as space charge effects and extend of field deviation from ideal profile. We address these factors and reports acquired dependencies. To reach high performance in these type of analyzers we need to choose an appropriate trap geometry, injection ion beam parameters, and create an electrostatic field of acceptable quality. Also, the influence of space charge effects should be removed or compensated.
We used the 3-dimensional particle in cell approach [2] to calculate the net field of the ion trap perturbed by the field from large ion population and to integrate motion of individual charged particles. The effective electric field was calculated as a superposition of the analytictrap field and ion fields' calculated via the 3-dimensional Poisson equation. Four different published orbitrap geometries were considered : geometry close to preproduction orbitrap, close to standard orbitrap, close to high field orbitrap and close to compact high field orbitrap.
Limits of resolution caused by trapping field non-harmonicity and the magnitude of frequency shift as a function of the number of charged particles in ion cloud are determined. Dependences of the measured ion frequency on the number of ions for orbitraps with different field curvature and different average axial amplitude were obtained.
We also addressed the ion peaks coalescence phenomenon in different orbitraps geometries. It was shown that for a higher field curvature peaks coalescence occurs at a larger number of ions in a cloud.
Influence of the electrostatic field imperfections caused by surface geometry deviation from the ideal profile was analyzed. It was shown that such geometry deviations cause signal decay and resolution degradation. A critical minimum level of surface deviation from the ideal profile was determined. The influence of voltage instability on ion motion was considered as well.
All simulations were performed on "GraphIT! " supercomputer of Moscow State University. The authors acknowledge the support from the Russian Foundation of Basic Research (grant 10-04-13306) , from the Russian Federal Program (state contracts 14.740.11.0755, 16.740.11.0369) , and from the Fundamental Sciences for Medicine Program of the Russian Academy of Sciences.

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PTu-115
11:10 – 12:20

Characteristics of a laser assisted vacuum-type electrospray droplet beam source

Satoshi Ninomiya, Lee Chuin Chen, Yuji Sakai, Kenzo Hiraoka

University of Yamanashi, Kofu, Japan

Keywords:
Electrospray, Laser, Massive cluster, SIMS

Novel aspects:
A technique for stable vacuum electrospray of volatile liquids was developed as a high-brightness massive cluster beam source.

Abstract:
One of the most serious problems of secondary ion mass spectrometry (SIMS) is its low sensitivity to organic molecules. The sensitivity has been increased by using cluster probes such as SF_5^+, C_{60}^+, Au_3^+ and Bi_5^+, and improvement of spatial resolution on imaging mass spectrometry for real world organic samples with these cluster ions has been reported. However, secondary ion emission yield in SIMS is still not enough for high resolution (< 1 micron) imaging, and there is a pressing need to improve the sensitivity. Gas cluster and charged-droplet beams are good candidates for next generation cluster-SIMS probes. Compact gas cluster ion guns are now commercially available for etching in surface science. In our laboratory, electrospray droplet impact (EDI) method based on ambient electrospray technique has been developed as a new massive cluster ion beam source, and it has been very successful in achieving efficient ionization of organic molecules and etching of polymers and metal oxides with leaving little damage. However, it currently lacks adequate beam focusing and beam density to achieve imaging with sufficient spatial resolution and within a reasonable time. To solve these problems, we propose a new method of producing a high-brightness charged-droplet beam, and have developed a technique for producing stable electrospray of volatile liquids (especially water) under vacuum. It has been known that vacuum electrospray of volatile liquids is extremely difficult because of two major problems : (1) the occurrence of electric discharge under low vacuum conditions and (2) the freezing of the volatile liquids introduced into the vacuum by evaporative cooling. To prevent the electric discharge, the experimental chamber pressure was significantly improved by using turbomolecular, mechanical booster and rotary pumps. To prevent freezing of the liquids introduced in vacuum by evaporative cooling, the tip of the electrospray emitter was heated by infrared laser. This vacuum electrospray technique can be expected to be a high-brightness massive cluster ion beam source. In this paper, the characteristics of a vacuum-type electrospray droplet beam source as a SIMS probe will be presented.

PTu-116
13:30 – 14:40

New plasma-based ion source mass spectrometer (ECRIS-MS) and its application to rapid detection of volatile compounds

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[1]RIKEN, [2]National Research Institute of Police Science

Keywords:
ECR, plasma, CWA, volatile, in-situ

Novel aspects:
A new plasma-based MS was customized. Its size is considerably small. Volatile compounds can be readily and stably detected, which allows the on-site detection of chemical warfare agents.

Abstract:

[Purpose]

We have been developed new mass spectrometer with plasma ion-source based on "electron cyclotron resonance ion source" (ECRIS). Since ECR plasma has high electron density and energy, sample compounds introduced into ECR plasma are efficiently and stably ionized and then detected as their decomposed forms such as atoms and atomic groups.

In this session, we will show a new version of MS with ECRIS, "miniECRIS-MS". Unlike other ECRIS typically used in accelerator, the size of its ion source chamber is considerably small, which allows sample monitoring *in-situ*. As an example, several volatile chemical warfare agents (CWA) in air were measured with miniECRIS-MS. And the characteristics and potentials of miniECRIS-MS for rapid detection of volatile compounds will be discussed.

[Equipment]

The miniECRIS-MS consists of three parts: 1) ECR plasma chamber made of permanent magnet (cylindrical shape; 10 cm in diameter and 15 cm in length), 2) quadrupole mass analyzer and 3) continuous electron multiplier. ECR plasma was generated by feeding air occasionally including volatile compounds into plasma chamber under micro-wave radiation.

[Sample measurement]

1. Air: atmospheric air was measured as a background of CWA sample. Pure gases (N_2, O_2, Ne, Ar, Kr, Xe and mix gas) were also measured. 2. Simulant: dichloromethane (CH_2Cl_2: DCM) was volatilized in a glass jacket containing atmospheric air. Then DCM with air was introduced into plasma chamber through fused silica capillary. 3.CWA: several CWAs, such as sarin ($C_4H_{10}FO_2P$), mustard gas ($C_4H_8Cl_2S$) and phosgene ($COCl_2$) were measured. The sample introduction into the chamber was the same as the case of simulant.

For each sample, mass spectra were obtained with varying experimental conditions (pressure of sample gas in the plasma chamber, RF power, RF frequency, etc.)

[Results and discussion]

1. Air: many peaks derived from air components were observed in the spectra. Main peaks were from nitrogen molecules (N_2^+, N^+ and N^{2+}) and oxygen molecules (O_2^+,$O^+$and O^{2+}). Other peaks from carbon dioxide (CO_2^+), argon (Ar^+, Ar^{2+}), water (H_2O^+, OH^+) were also observed. In addition, a peak not corresponding to air components was observed at *m/z* 30. Since the same peak was also observed in N_2 -O_2 mix gas, the peak is considered to be NO^+ generated by plasma radical reaction.

2. Simulant: when RF power was high (10 W), DCM was almost completely decomposed and detected as single atoms such as C^+, Cl^+. This indicates that miniECRIS-MS can be used as elemental analyzer in high RF power mode. With decreasing RF power, fragment ions (*e.g.* CH_2Cl^+ *m/z* 49) and even molecular ion ($CH_2Cl_2^+$ *m/z* 84) were observed, showing that miniECRIS-MS can also provide information on the structure of the analyte.

3. CWA: in this measurement, we focused our attention on the detection of CWA as single atoms. In other words, miniECRIS-MS was operated in high RF power mode. Characteristic elements underlined in the formula were detected from sarin ($C_4H_{10}\underline{F}O_2\underline{P}$), phosgene ($CO\underline{Cl}_2$), mustard gas ($C_4H_8\underline{Cl}_2S$) and the other CWAs. Each CWA had optimum RF power region required for the efficient decomposition and ionization. For example, Cl^+ was observed both from phosgene and mustard gas. However, phosgene required more RF power than mustard gas. This would reflect the chemical environment around Cl atom in each CWA.

[Conclusion]

A prototype miniECRIS-MS was assembled. Various CWAs were observed within one minute. This indicates the potential of miniECRIS-MS for the rapid detection of volatile compounds, and leads to the comprehensive and real-time detection of CWAs as countermeasures against chemical terrorism. We will further improve the equipment for hogher sensitivity and identification ability. The detail of portable-type miniECRIS-MS shall be introduced in the presentation.

PTu-117 Adaptive Noise reduction in MALDI TOF Mass Spectrometers

11:10 – 12:20

Ian Brookhouse, Andrew R Bowdler, Ian Sherwood

Kratos Analytical Ltd.,Manchester,England

Keywords:
MALDI-TOF Adaptive Noise Reduction Electronics

Novel aspects:
An adaptive noise reduction technique enabled by advances in high performance electronics utilising quasi concurrent measurement of noise and time of flight data.

Abstract:
Fundamental to the operation of modern MALDI TOF mass spectrometers is the need for digital and analogue electronics all running at GHz speeds. In the mass spectrometer and TOF analyser, high-voltage switches capable of switching many kilovolts in nanoseconds provide the pulses for pulsed extraction and ion gating. Transient recorders in MALDI TOF instruments are typically required to digitise the analogue signal from the ion detector at sample rates of 2 GHz or more, up to around a millisecond long with a resolution of 8 bits and repetition rates around 1 kHz. Furthermore, many transients are usually accumulated in the recorder before the final TOF spectrum is transferred to the computer for analysis and storage. There is also a need for high speed and precision triggering to fire the laser and to provide the various pulses as mentioned above. These requirements are not satisfied by commercial digital oscilloscopes, which means that purpose designed electronics have to be produced, often by the instrument manufacturers themselves. In general the resultant design is a variety of very high speed digital electronics including the digitiser itself but mostly comprising the transient recorder memory.

A typical MALDI TOF MS (or MS/MS) spectrum will be the summation of many individual laser shots and the resulting TOF transients in order to average out the random noise in the mass spectrometer. In this way the signal to noise can be maximised and the smallest peaks in the spectrum detected. However, the accumulation and averaging does not reduce the effect of any systematic noise and ultimately the signal to noise limit can depend on the size of such artefacts present either in the mass spectrometer electronics or in the digitiser. For the MALDI TOF MS the potential for systematic electronics noise is very significant. As well as obvious design measures such as the shielding of electronics and power supplies, there are some simple ways to reduce the effect of systematic noise such as randomizing the start time of the transient in the digitiser memory.

We will present another approach to reducing systematic noise in TOF spectra to the point where the effect of the noise is negligible. The principle is that the noise data is acquired, ideally during the time that the signal data is acquired and the resultant noise spectrum is then subtracted from the signal spectrum. The subtraction can be carried out during or after acquisition and by the electronics or by software. Because the noise acquisition produces essentially the same data but without the MALDI ion signal, the systematic noise can be reduced to near zero. By repeating this process many times during the data acquisition process noise that has low frequency variation may be removed in an adaptive manner. Examples will be presented of MALDI TOF MS data before and after the reduction of different types of systematic noise. In each case both the signal spectra and the noise spectra will be shown. It will be demonstrated that, by this method the signal to noise ratio can be improved to the point where it is effectively limited by the random electronic or chemical noise in the mass spectrometer.

PTu-118
13:30 – 14:40

Development of an ion transmission enhanced tandem ion guide system for triple quadruple mass spectrometer

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Shimadzu Co., Kyoto, Japan

Keywords:
Triple quadrupole, high speed, high sensitivity, neutral loss scan

Novel aspects:
Development of a higher ion transmission tandem RF ion guide system to enhance scan sensitivity

Abstract:

Introduction

The mass spectrometer coupled to atmospheric pressure ionization source needs to have differential pumping system in order to maintain high vacuum in analyzer part. Commercial triple quadrupole mass spectrometer typically has from 3 to 4 differential pumping stages and the RF ion guide was generally utilized as an ion focusing device in higher pressure region of ion path. The ions generated under atmospheric pressure needs to be efficiently focused to minimize the loss of ion prior to introducing into quadrupole analyzer by using RF lens system. Using a QqQ which has 4 differential pumping stages, we have investigated fringing field of RF ion guide installed in second and third differential pumping stage and finally the sensitivity was improved 5 times.

Methods

Three distinct designs of the tandem quadrupole RF ion guide in differential pumping stages have been investigated. In order to examine the characteristics and performance of these configurations, each RF ion guides were installed in a triple quadrupole mass spectrometer (LCMS-8030, Shimadzu Corporation, Kyoto, Japan) . Each tandem RF ion guide systems have their inscribed radius a) 1.5mm, b) 2.0mm and c) 2.8mm and the system has one DC lens between RF ion guides, and its diameter is 4 mm.

Preliminary Data

The optimum operating RF voltage of quadrupole ion guides was acquired experimentally. The voltages were 100V, 50V and 27V for r_0= 2.8, 2.0 and 1.5 respectively. Then each pseudo potential is calculated by the following equation,

$V^*(r) = (4qV^2/m\Omega^2 r_0^4) r^2$

where, r_0 is the radius of the inscribed circle of the ion guide, V is the amplitude of the RF voltage, m is the mass of the ion, and q is the electric charge. The calculated pseudo potential of each ion guides is almost same at m/z 168. In other words, ion focusing effect inside of the RF ion guide is almost identical. We have simulated the fringing focusing field of smaller r_0 ion guides was stronger than that of larger r_0. Therefore we have achieved higher sensitivity using smaller r 0 ion guides. We have replaced the tandem quadrupole ion guide (r_0= 2.0) system with existing tandem octapole ion guide system of an ultra fast TQ mass spectrometer (LCMS-8030, Shimadzu Corporation, Japan) . We acquired Q 1 scan spectra of pesticides at three different scan speed with flow injection analysis. (5000, 10000 and 15000 u/sec) . Besides the nature of quadrupole ion guide which is narrower mass range transmission comparing to other ion guide such as hexapole and octopole, the absolute intensity of Q 1 scan with all three scan speed was increased 2 to 5 times.

PTu-119 The A-Wave principle: new way to design ion guides and similar devices

11:10 – 12:20

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Keywords:
ion guides, A-Wave, T-Wave, RF fields, pseudopotentials

Novel aspects:
A new way to desing the ion guides, etc., with the pseudopotential wave which traps ions and moves them synchronically in space, is considered.

Abstract:
The modern mass spectrometry widely uses the devices based on radio frequency electric fields, including that where the charged particles move in gas-filled media : ion traps, quadrupole mass analyzers, gas-filled cooling cells, collision cells, ion guides, ion funnels, Q-arrays, etc. These and similar devices can be found in nearly each commercial or experimental mass spectrometers with atmospheric pressure ionization. The effectiveness of these devices influences the effectiveness of the mass spectrometer greatly.

The movement of charged particles in radio frequency (RF) electric fields is described qualitatively with good accuracy by the pseudopotential theory [1] . However, this useful theoretical approach deals mainly with the stationary pseudopotentials, the latter results from the nature of RF voltages used to excite the RF electric fields. General theory [2] overcomes this limitation and enables to create the devices where the movement of the charged particles is controlled by time-dependent pseudopotentials with the *a priory* specified properties. While some particular examples of RF electric fields with time-dependent pseudopotentials were already used before [3] , only general theory enables to utilize this tool to full extent. (However, if somebody wants to look deeply for the predecessors by priority, these will be Sir Isaac Newton, James Clerk Maxwell, Michael Faraday and Alessandro Guiseppe Antonio Anastasio Volta, like for any other device relevant to the charged particle optics.) In many cases the flexibility to control the movement of the charged particles by this new tool enables to expand significantly the properties of the corresponding mass spectrometric devices [5] .

This presentation considers a specific case of the RF electric fields with time-dependent pseudopotentials which were called by the author 'the fields with the Archimedean properties', or the A-Wave fields. These RF fields are characterized by a pseudopotential wave composed from alternating maxima and minima, where the said maxima and minima move along the axis of the device following the predefined law. The charged particles are trapped by local pseudopotential wells corresponding to pseudopotential minima, are compressed into spatially separated and isolated packages, are moved synchronically with the said pseudopotential wave independent of their masses, charges and to some extent the initial velocities. The group velocity of the packages is totally controlled by the applied RF voltages and can vary between units of Hz to hundreds of kHz. In most cases the corresponding device is formed by a periodical sequence of the electrodes like that used for the T-Wave™ ion guides [4] , however, the power supply is strongly different for the A-Wave transport as compared with the T-Wave™ transport. The usage of non-periodic A-Wave structures and local manipulations with the individual trapping zones enables us more possibilities to manipulate the trapped ions while their transportation.

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[2] A.S.Berdnikov, The time-dependent pseudopotential and its application for description of the averaged motion of the charged particles, *Nauchnoye priborostroenie*, vol. **21** (2011) No 2, p. 77-89 ; vol. **21** (2011) No 3, p. 83-96 ; vol. **21** (2011) No 4, p. 75-85 ; vol. **21** (2011) No 4, p. 86-102 (in Russian) .
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PTu-120
13:30 – 14:40

COUPLING OF AN INERT ION CHROMATOGRAPHIC SYSTEM WITH ICP-Q-MS FOR ROBUST AND ACCURATE ELEMENTAL SPECIATION

Tomoko Vincent[1], Daniel Kutscher[1], Lothar Rottmann[1], Julian Wills[1], Shona McSheehy[1], Detlef Jenen[2]

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Keywords:
iCAP Q, ICP-Q-MS, QCell, Speciation, Dionex,

Novel aspects:
The powerful new Thermo Scientific iCAP Q ICP-Q-MS with innovative QCell technology, coupled with Dionex HPLC, can effectively remove all kinds of spectral interferences whilst maintaining high detection sensitivity.

Abstract:
Speciation of metals and other trace elements becomes more and more important to accurately address the toxicity or other potential hazards of a given sample. Many physicochemical parameters such as toxicity, reactivity and mobility are strongly dependent of the elemental species, so that detailed information on the chemical form of an element is important rather than the total concentration of the element itself.

A new package for speciation is used to demonstrate the rapid and accurate analysis of sub-ppb levels of elemental compounds, including examples like hexavalent chromium, that is under regulation in drinking waters, or arsenic species in food. Especially the latter application has gained attention lately, since it was reported that ingredients used in toddler formula may contain more than 100 ng g^{-1} of As being present predominately in its toxic inorganic species, As (III) and As (V) .

The Dionex ICS-5000 ion chromatography system hyphenated to the Thermo Scientific iCAP Q ICP-Q-MS is a powerful tool for the determination of the species distribution of an element within different samples. Due to its completely metal-free solvent path, instrumental background can be reduced, and also aggressive mobile phases such as acids can be used. A variety of analytical columns with outstanding separation capabilities enable the reduction of analysis time and solvent consumption together with excellent separation performance. The new iCAP Q ICP-Q-MS with innovative QCell can effectively remove all kinds of spectral interferences while maintaining high detection sensitivity. Therefore, also low concentrated species can be detected and accurately quantified.

With this combination unattended routine speciation analysis is possible with simple hardware coupling and by use of a new software platform fully integrated control, dedicated chromatographic integration, data processing and compound specific QC is offered.

PTu-121
11:10 – 12:20

A Two-Dimensional Particle Tracking Velocimetry Investigation for Visualization of Gas Flow inside the Ion Funnel

Diamantis Kounadis[1], Dimitris Papanastasiou[1], Ioannis Orfanopoulos[1], Alexander Lekkas[1], Ioannis Nikolos[2], Emmanuel Raptakis[1]

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Keywords:
ion funnel, vacuum fluid dynamics, Particle tracking velocimetry

Novel aspects:
A novel method for studying and optimising gas flow dynamics with reliable experimental methods and its application to ion funnel aerodynamics.

Abstract:
Vacuum interface design and intermediate pressure ion optics are critical aspects for the development of high performance mass spectrometers. The ion funnel is one of the most recent technological advancements in the field with a significant impact on instrument sensitivity. Characterization of the ion funnel has been mainly performed experimentally under normal operating conditions of a mass spectrometer and to some extent by ion optical simulations. Information about the gas dynamical effects present in the device and the effect on ion transmission has not yet been presented. Here, an experimental investigation of the gas dynamics inside the ion funnel is performed based on the Particle Tracking Velocimetry (PTV) method. A spark discharge particle source is developed to generate tracer nanoparticles capable of scattering laser light. The nitrogen flow is seeded with the tracers and a CCD camera is used to capture the light which produces an average 2 D velocity vector field along the plane of the laser sheet. The spark discharge source designed and constructed for the purposes of the experiment is capable of producing both mono-disperse and poly-disperse particles and has been extensively characterized by a Differential Mobility Analyzer (DMA). A prototype ion funnel assembly was designed using CAD software and constructed from a composite material using 3 D color printing with a resolution of 300x450 dpi which produced a model with accuracy of less than 0.1 mm. The thickness of the electrodes is 0.6 mm and an exit aperture of 2.5 mm was used. Inserts are constructed along the length of the funnel and sealed with glass windows which allow for optical access with a laser and a CCD camera. The model is introduced in the first vacuum compartment of a vacuum chamber equipped with a capillary inlet with 0.5 mm id. The ion funnel model is mounted on a supporting base and positioned 20 mm downstream the outlet of the capillary interface. A 33 m^3/hr mechanical pump is used to evacuate the first vacuum compartment to 30 mbar. The second vacuum compartment evacuated to 2 mbar using a second mechanical pump is in direct communication with the first compartment through a 2 mm end aperture positioned at the end of the funnel. A 1 mm thick laser sheet is introduced through a side slot running along the length of the funnel. An exit slot of equal length allows for the laser light to exit the device and reduce background noise on the CCD. Laser light scattered on graphite nanoparticles is observed through optical ports with dimensions of 12x12 mm^2 near the entrance while smaller size ports are used toward the exit of the device. Preliminary results show that the formation of the free jet remains undisturbed at the entrance of the funnel and image processing shows particles with velocities exceeding 400 m/s. Jet boundaries are confined to within ± 2 mm during the first 20-30 mm from the entrance. The velocity of the gas drops to below 300 m/s at 50 mm distance relative to the entrance, a region where the onset of jet instabilities occurs. Gas turbulence is observed further downstream and the average flow field shows particles moving in all directions at speeds as low as 30 m/s. Experimental results obtained by the PTV method provide significant insights into the gas dynamical aspects related to the design of the ion funnel design.

PTu-122 Source Cleaning in Maldi Mass Spectrometers by UV Laser Desorption

13:30 – 14:40

John M Allison, Andrew Eaton

Kratos Analytical Ltd

Keywords:
MALDI, Source Cleaning, UV Laser

Novel aspects:
The same UV laser is used for MALDI and to clean the ion source. Most efficient method for the de-contamination of MALDI ion source electrodes.

Abstract:
The MALDI process facilitates the vaporization and ionization of biomolecules and large organic molecules. The molecules are embedded in a matrix which absorbs UV light. When a UV laser is fired on the sample spot to initiate the MALDI process a plume of ionised and neutral analyte and matrix molecules is ejected from the sample plate. The ionised molecules are accelerated away through apertures in the electrodes by an electric field formed between the electrodes. However, much of the matrix material, and some of the analyte, is not ionized and this neutral plume continues to expand from the sample spot until it is deposited on surfaces in the vicinity of the ion source. The contamination builds up over time until at some point an insulating layer is formed that charges up and adversely affects the operation of the ion source. At this point the source electrodes must be cleaned.

For many years the principal method of cleaning vacuum MALDI ion sources was to vent and open the source housing to enable the first electrode to be cleaned in situ or the source removed completely for thorough cleaning. In both cases, in addition to the cleaning time, several hours were required to restore the vacuum once the housing was closed and perform the required HV conditioning, instrument tuning and mass calibration procedures. In many applications of biochemistry there is a growing requirement for higher throughput machines, which can now be realised by the introduction of instruments capable of running at repetition rates of over 1 kHz. This has increased the rate of contamination build up on source electrodes, and the frequency with which they must be cleaned, to such an extent that it is no longer practical to vent the instrument every time the source requires cleaning. Thus there is a requirement to find an effective method to clean the electrodes that does not require the source housing to be vented.

Methods not requiring the source housing to be vented have been described that heat the electrodes up to temperatures of 250°C using contact heaters or IR laser radiation. The effectiveness of heating the source electrodes is variable and depends very much on the matrix used and the amount of contamination present, with thin layers being much easier to remove than relatively thick layers that can build up even in a relatively short time. Further, some contaminants, particularly polymers, can be very difficult to remove simply by heating.

In this paper, a novel effective method for automatically removing contamination from apertured electrodes will be presented. The method exploits the fact that most of the contamination on the source electrodes was originally desorbed from the sample spot using UV laser irradiation and thus will be readily desorbed from the source electrodes if it is again irradiated with UV light of sufficient energy density. This method uses the MALDI pulsed laser already employed in the instrument with scanning reflective optics coupling the laser energy directly into the contamination to be removed, which leads to very effective and rapid desorption of the material from the surface. Crucially the method avoids significant heating of the electrodes, thus avoiding shortcomings associated with other techniques. The method enables fully automatic, rapid and very efficient source electrode cleaning to be carried out without the need for source removal, venting or even removal of the sample plate from the instrument. This method has proven to be effective in cleaning all common matrixes from source electrodes even when an appreciable layer of contamination has been allowed to build up.

PTu-123

11:10 – 12:20

Compensating for Space Charge Effects and Increasing the Mass Accuracy and Dynamic Range of Quadrupole Ion Trap Mass Spectrometers

Jae Schwartz, Philip M Remes

Thermo Fisher Scientific, San Jose USA/CA

Keywords:
Space Charge Effects, Quadrupole Ion Trap, Dynamic Range

Novel aspects:
A space charge calibration process can increase the mass accuracy and dynamic range of quadrupole ion trap mass spectrometers.

Abstract:
Ion trap mass spectrometers are well-regarded in the analytical instrument field for their sensitivity ; however, their inherent dynamic range can certainly be improved. While single ions can be detected, the upper limit of useful ion capacity is bound by ion-ion interactions. These interactions induce shifts in observed mass positions, and ultimately cause these mass spectral peaks to become degraded (broad and less intense) . Practically, the ion limit is set well below this point, corresponding to some arbitrary maximum value of mass shift. However, such a limit can sacrifice dynamic range for mass accuracy, even though substantial capacity is available before complete peak degradation. A method has been developed to calibrate the mass shifts in the range before degradation, extending dynamic range while maintaining mass accuracy.

A modified LTQ Velos linear ion trap mass spectrometer was used for these studies. LTQ Calmix, and polyethylene glycol with average molecular weights 200, 400, 600, and 1000 Da were used as standards. The relationship between number of ions and mass shift was calibrated and used to adjust the observed mass position of subsequent spectra. Numerical simulations were used to support the experimental results.

Previous efforts to calibrate space charge mass shifts were useful only in very simple or too-specific instances, but never in the case of an unknown, arbitrarily complex spectra. The difficulty arises because the influence of other ions on an ion of interest depends on their proximity in a complex way. However, our results show that the interactions with the other ions accumulate linearly. Therefore, once the relationship between proximity and mass shift is known, the effects of other ions can be predicted based on their intensity and proximity to the ion of interest. The total effect of the other ions in the trap on an ion of interest is the linear combination of their individual influences. A method of selectively accumulating ions of various intensity and proximity to a calibrant was used to perform a calibration of the space charge effect, which can then be used to adjust the mass assignments. In preliminary results, the method was able to correct for the space charge shifts in MS/MS spectra of 30,000 total ions, where product ions were shifted by 0 -0.15 Da. This represents a 3 x increase over the typical target total ion abundance of 10,000 ions. The actual new spectral space charge limit will be explored in more detail. Numerical simulations will also be compared with the experimental results, and a model for the influence versus proximity relationship will be proposed.

PTu-124
13:30 – 14:40

Further expansion of the MCP ion detector output linearity using a current buffering concept.

ETSUO IIZUKA, MOTOHIRO SUYAMA, TOSHIYUKI UCHIYAMA, YUYA WASHIYAMA, KATSUYUKI ISHIGURO

HAMAMATSU PHOTONICS K. K., IWATA, JAPAN

Keywords:
Ion detector, MCP, Linearity, Dynamic range, Low aspect

Novel aspects:
Increase output linearity of MCP ion detector is achieved by low-aspect-ratio current buffering MCP concept.

Abstract:
Microchannel Plate (MCP) ion detectors are widely used for TOF-MS because of their fast time response less than 1 ns, a large effective diameter up to 77mm, and the flatness within 20 micron. However, the output charge saturation usually occurs at lower levels compared to the discrete-dynode electron multiplier tubes, which limits its usefulness in some applications. The upper limit is restricted by the resistance of the MCP, which regulates strip current. When the resistance is larger, the flow of charge is inhibited at higher outputs limiting the output linearity.

We have been studying MCP materials to lower its resistance, as well as an appropriate aspect ratio of MCP channels to achieve the higher output currents, and found the current buffering concept worked well. In this concept, a new MCP with low-aspect-ratio (LAR) , length per diameter, of 20 compared to the conventional specification of 40 to 60 is utilized as an additional multiplication stage to increase the electron output from the two stage MCPs. The low resistance LAR MCP can operate without melting because it requires half the applied voltage, thus half the power consumption a conventional unit would need. Since the gain of a LAR MCP is low, this is used as a current buffer following the two stage MCPs.

Using the current buffering concept, we fabricated a LAR MCP, where the aspect ratios were 20, using the same process and materials to achieve the lower resistance, which was 0.25M ohm. The strip current was 1 mA at the applied voltage of 250V. Under this condition, the power consumption was 0.25W, and we confirmed stable operation while obtaining a gain of 10. This MCP was combined with a 2 stage MCP, creating a 3 stage MCP, and tested as an MCP ion detector. This combination resulted in an upper output current limit of 12uA, which is 2 times higher than our lowest resistance MCP detector, but not as high as our expectation of 70uA .

We have investigated one limitation of the linearity, and tried to optimize the performance. As a result, we found a potential difference of 500V between the second and the third (current buffering) MCP helps to increase the linearity up to 30uA. Since this potential increases the gain of the third MCP, it is suggested that the linearity of the second MCP limited the overall performance of the former configuration. We are sure that there is another limiting factor, thus we are still carrying out additional research.

In an attempt to optimize our detector design, we are designing a one stage MCP with a high aspect ratio that should obtain the same gain as our initial two stage MCPs. This should achieve the same results with a simpler configuration. The progress of this optimization will be presented in our paper.

PTu-125
11:10 – 12:20

Sensitivity improvement of MCP-based Ion Detectors for Mass Spectrometry

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Keywords:
microchannel plates, effective open-area ratio, ion detection sensitivity, pulse height distributions,

Novel aspects:
The large OAR MCP is effective to increase the detection sensitivity.

Abstract:

Introduction

Microchannel plates (MCPs) are widely used as ion detectors in mass spectrometry because of large effective areas, flat detection surfaces, and fast response times. The signal generation in MCP is triggered by ion-impact-induced secondary electron emission at the inner wall of each channel. The secondary electrons are accelerated and impinge on the channel wall again, which generate additional secondary electrons. This electron cascade multiplys the number of electrons, which are collected at an anode and generate an output pulse signal. Sensitivity of MCP is related to (a) the ion-electron conversion efficiency, which is the expected number of secondary electrons generated by the initial impact of an ion on the channel wall, and (b) the effective open-area ratio (OAR) , which is defined as the ratio of the channel opening space to the total MCP surface area. The upper limit of ion counting rate and pulse height is affected by (c) the detector saturation, which is caused by a large amount of the electron cascade current that preclude proper operation by the charge accumulation near the channel exit.

We improved the sensitivity of MCPs by such methods as channel surface coating with low work function materials to increase the ion-electron conversion efficiency, resistance reduction to increase the upper limit of the electron cascade current, and OAR increase by etching the channel walls on the input side of MCPs. This poster reports on the effect of OAR on ion detection sensitivity quantitatively.

Experimental

The ion detection sensitivity was studied by a double-focusing mass spectrometer (JEOL JMS-600) . The response of the MCP-based ion detectorss to individual single ion impacts was measured with the ions of Ar and L-cysteine generated by electron ionization (EI) at 70 eV. These ions were accelerated by a static high voltage of 3 kV. The *m/z*-specified ions were selected by the electric and magnetic sectors, and impinged on the MCP surfaces. We prepared two types of MCPs with different OAR values : 57% (S-MCP) and 75% (N-MCP) . These MCPs were interchangeable in vacuum by manipulating the detector assembly. A comparison of the ion detection sensitivity between the two MCPs was made by recording pulse height distributions with a multichannel analyzer.

Results and Discussions

The peak channels of pulse height distributions measured for Arions were found to be irrespective of OAR. The ion count rate of N-MCP for Ar ions was 15% higher than that of S-MCP. This difference of the count rates is almost equal to that of the OAR values.

We also studied the effect of molecular species on pulse height distributions. The peak channel position measured for the L-cysteineions became low. The peak position shift is reasonable with a mass dependence of the number of initial secondary electrons. Since the lower peak channels resulted in the increase of miscounting at a fixed discrimination level, the difference of the count rates between the two MCPs became small : 6 % for L-cysteine ions. For both ions, it is demonstrated that our fabrication method for the large OAR MCP is effective to increase the detection sensitivity.

PTu-126 Round Focusing Magnetic Prisms for Surface Treatment Devices

13:30 – 14:40

Valentina A Mikhalevich, Viacheslav D Sachenko

Institute for Analytical Instrumentation of RAS, St.Petersburg, Russia

Keywords:
magnetic prism, sector field, fringing field, round focusing, mass-spectroscopic imaging

Novel aspects:
novel compound magnetic prism geometries and novel design ideas for energy- and/or mass-spectroscopic imaging devices

Abstract:
Static magnetic prisms known in electron optics as *magnetic sector fields* are important elements of many research devices. As mass/energy analyzers, or as separators of the opposite-directed beams for the image aberration correction by a mirror, or for the direct illuminating the specimen, the magnetic prisms are essential part of any modern surface treatment devices intended for a study and a control of morphology and crystal structure, and chemical analysis of the micro- and nano-scale areas of a solid surface.

It is well known that optical properties of the magnetic prisms in the radial and axial (vertical) directions are quite different by nature. In the radial direction, the magnetic homogeneous field of the prism acts like a thin planar lens located at the prism bisector plane. In the vertical direction, the focusing action of the homogeneous field is absent. However, in case of the tilted boundaries of the prism, the vertical focusing action appears due to the well-known "*edge field effect* " which depends on the inclination angle of each boundary. Appearing here "*edge lenses* " act like quadrupole lenses. Therefore, the magnetic prism acts in this case like a multi-quadrupole system producing an axial astigmatism and an image ellipticity when the image beam passes through the prism.

The key goal of our investigations is to find the reasonable geometries of the magnetic prisms which allow to provide the simultaneous correction of the astigmatism and the image ellipticity for any positions of the object plane. This special type of the focusing is called by the authors a *round focusing*, and this focusing for the magnetic prisms with the planar-parallel active pole pieces is considered here. It can be shown the singular prism with the homogeneous field cannot produce the round focusing. Therefore, the specific magnetic prisms, consisting of three differently excited magnetic segments being geometrically symmetric regarding the prism bisector plane, by the equal excitations of the outer segments, are considered here. That symmetric construction, invented by L. Veneklasen, allows to produce the stigmatic focusing (vertical focusing occurs here by passing the image beam through the fringing fields between the outer and inner magnetic segments) . However, to produce the round focusing it is necessary to optimize the geometrical sizes of the outer and inner magnetic segments, the intermediate gaps, and the excitation ratio between the outer and inner segments.

In the frame of well-known "SCOFF " model for the fringing fields between the outer and inner segments, it is found the prisms under consideration can provide the round focusing for the different deflection angles by the excitation ratio of about 3. However, by taking into account the extended fringing field distribution between the magnetic segments, it is found the optimal excitation ratio depends on the deflection angle to a greater extent, and increases noticeably. Additionally, the 3rd order vertical aberration coefficient is varied strongly here. Both these effects are the result of the weak "*fringing field lens* " action which could not be analyzed in the "SCOFF " model. Using the "*extended fringing field* "model of the prism, the authors have discovered the reasonable geometries providing the round focusing for the prism deflection angles 90° and 60°. One of these geometries was verified experimentally, and has demonstrated a very satisfactory confirmation of the theoretical predictions.

The results of investigations of the round-focusing magnetic prisms, and the novel design ideas for energy- and mass-spectroscopic imaging devices are presented.

PTu-127
11:10 – 12:20

From conventional Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) to a universal trace gas analyzer

Lukas Märk[1], Alfons Jordan[1], Achim Edtbauer[1], Eugen Hartungen[1], Simone Jürschik[1], Philipp Sulzer[1], Tilmann D Märk[1,2]

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Keywords:
PTR-MS, proton transfer reaction mass spectrometry, charge transfer, trace gas analysis, PTR-TOFMS

Novel aspects:
Reagent ions in PTR-MS are extended to Kr+ and Xe+. This has never been done before and allows for the analysis of completely new substance classes.

Abstract:
Proton-Transfer-Reaction-Mass Spectrometry (PTR-MS) is a well established technology for real-time trace gas analysis in the fields of environmental research, food and flavor science, medicine, homeland security, etc. However, one limitation of conventional PTR-MS is that only compounds possessing a higher proton affinity than water can be ionized. Also recent add-ons, like the so-called Switchable Reagent Ions (SRI) feature, where O_2^+ (and NO^+) can be utilized to ionize trace gas molecules via charge transfer, are limited to compounds having ionization energies below 12.1 eV, which still excludes important substances like CO, CO_2, SO_2, CH_4, etc.
Here we present a novel development avoiding these limitations while preserving all advantages of conventional PTR-MS (fast response times, high sensitivity, no sample treatment, etc.) .
The setup of a typical PTR-MS instrument has been discussed extensively in literature. For the present innovation, where we introduce Kr^+ and Xe^+ as additional reagent ions (SRI+) , the principal instrumental setup stays the same. This means that even in the modified version no mass filter is necessary between the ion source, that is generating the reagent ions and the drift tube, where the ionization of the trace compounds take place. Pure Kr or Xe from an external gas cylinder is introduced into the ion source where Kr^+ or Xe^+ is formed, respectively. These reagent ions are subsequently introduced into the adjacent drift tube where they can interact with air containing traces of the sample and an inert dilution gas.
Kr has an ionization energy (IE) of 14.0 eV and therefore allows to ionize via charge transfer reactions important molecules like CO, CO_2, NO_x, N_2O, SO_2, CH_4, etc. We present measurements of several certified gas standards, which include "typical " calibration gases used frequently in PTR-MS, like e.g. benzene and additionally the above-mentioned compounds, which could not be ionized and therefore detected with a PTR-MS setup so far. By amending a PTR-TOF 8000 to SRI+ we can additionally take advantage of the instrument's high mass resolution. In cigarette smoke we can clearly distinguish CO^+ on 27.99 m/z from N_2^+ (impurity from the ion source) on 28.01 m/z and from $C_2H_4^+$ on 28.03 m/z.
CO_2 and N_2O are isobars with the masses 43.990 and 44.001 m/z, respectively. We analyzed a sample bag containing human breath (CO_2 source) enriched with N_2O from a gas cylinder. The results prove that the mass resolution is sufficient to identify the two compounds independently.
Xe has an IE of 12.1 eV, which is very similar to the IE of O_2. This means that in areas of application where oxygen cylinders are not allowed, Xe^+ can be utilized as a substitute, which is confirmed by our studies.
In summary, with SRI+ PTR-MS is now capable of switching between H_3O^+, O_2^+, NO^+, Kr^+ and Xe^+; thus there are virtually no limitations on substance classes and subsequently on fields of application anymore.
We gratefully acknowledge that this work was financially supported by the Austrian Research Promotion Agency FFG, Vienna. Patent pending (PCT/EP2011/064170) .

PTu-128
13:30 – 14:40

A Novel Precursor Isolation Method using Digital Ion Trap Mass Spectrometer

Kei Kodera, Masafumi Jinno, Kiyoshi Watanabe, Makoto Hazama, Masaji Furuta, Sadanori Sekiya, Kaori Kinoshita, Hidenori Takahashi, Kosuke Hosoi, Toshinori Kobayashi, Shinichi Iwamoto, Koichi Tanaka

Shimadzu Co., Kyoto, Japan

Keywords:
precursor isolation, MS/MS, Iontrap

Novel aspects:
We developed a novel highly effective precursor isolation method by which the yields of single unit peak were 90 % and 80 % for singly and doubly charged ions.

Abstract:
Mass spectrometry is an indispensable tool for proteomic research. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS) have been used for a comprehensive analysis of proteins since the 1990s, more recently, the importance of an in-depth analysis of post-translational modifications (PTM) is increasing. MS/MS and MS^n analysis on an ion trap mass spectrometer is a useful function for structural analysis of complex compounds such as oligosaccharides released from glycoproteins or biantennary glycopeptides.

To provide a more useful technology for MS^n, we have been working on the development of digital ion trap (DIT) mass spectrometer. DIT is driven by rectangular wave high voltages produced by high frequency switching of positive and negative high voltages whereas the conventional ion trap is driven by sinusoidal wave high voltages.

Since DIT has a higher ion capacity than the conventional ion trap at high mass range, it is well suited for the analysis of large molecules like PTM peptides. In addition, DIT has a feature that the frequency of trapping voltage can be changed instantly and arbitrarily.

Using this flexibility, digital asymmetric wave isolation (DAWI) method has been reported. DAWI is a unique method to isolate ions in DIT by the way of changing duty ratio of positive to negative phase of trapping rectangular high voltage. By using DAWI method, the ions of any mass ranges can be kept in the ion trap instantaneously with high yield. However, the "window size " of mass selection is not narrow enough and high mass cut-off in the window is not sharp, so that DAWI alone is not suitable for precursor selection for MS^n analysis.

In mass isolation analysis using the resonant excitation frequency scan, ion ejection can be provided with both ascending and descending order mass scan, so-called "forward scan " and "reverse scan ", respectively.

By the combination of forward and reverse mass scan, it is possible to keep the arbitrary ion peak for the precursor of MS^n analysis in the ion trap in which reported as single unit mass manipulation ion trap (SUMMIT) . However, this method takes long time to isolate ions and the yield was less than 50 % when a single unit mass ion, which is not the monisotopic ion, is selected from the monovalent ion isotope clusters. In this study, we have investigated the novel ion isolation method to achieve high resolution and high yield simultaneously.

Mass selectivity of filtered noise field (FNF) , resonance excitation, and DAWI were investigated by computer simulations.

Based on the optimal parameters determined by computer simulation, experiments for the single unit mass ion isolation were carried out using a home-built MALDI-DIT-TOFMS prototype. As a result, the yields of isolated ions with single unit mass were significantly improved to about 90 % and 80 % for singly and doubly charged ions, respectively. Here we suggest that this novel precursor isolation method is considered to be applicable to SUMMIT with high resolution and high yield.

PTu-129
11:10 – 12:20

A high accuracy FDM field solver for prediction of non-linear resonances in electrodynamic ion traps.

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Keywords:
FDM, SIMION, simulation, quadrupole, multipole

Novel aspects:
A high accuracy FDM field solver can give new insight into non-linear resonances in ion trap devices by simulation.

Abstract:
The finite difference method (FDM) is used widely for calculation of approximate solutions to the Laplace equation governing electrostatic and magnetic fields within bound systems, frequently using an iterative relaxation approach. FDM is commonly used due to its simplicity, speed and wide availability[1]. In mass spectrometry, FDM-based ion optical modelling of systems is commonly employed during instrument design and experimental interpretation.

A high accuracy FDM field solver incorporating accurate treatment of electrode boundaries[2] is described here, and a performance comparison to a commercial FDM solver is given. The high accuracy FDM algorithm is used to solve several electric fields for which analytical solutions may be calculated, and the accuracy is compared to a commercially available FDM field solver for several FDM grid densities.

For curvilinear electrodes, this 2nd order FDM algorithm incorporating accurate treatment of electrode boundaries typically results in greater accuracy than that obtained using the 2nd order FDM solver of a commercial software package, where the electrode boundaries are rounded to the nearest grid node position (using the same grid density in each case). The use of a similar 4th order FDM algorithm, also incorporating accurate treatment of the electrode boundaries, increases this accuracy by several orders. Although for the same grid sizes these more sophisticated algorithms require more arithmetical operations per individual grid node (resulting in greater calculation time), the size of the grid necessary to obtain some fixed accuracy is much less than that for conventional finite difference methods. The ability to employ sparse grids provides several advantages : it allows simulation of large systems whilst maintaining high accuracy when random access memory restrictions might otherwise limit the simulation, and provides a considerable reduction in computation time for field solving when solving to some fixed accuracy.

The high accuracy FDM field solver reported here is used for analysis of several example problems. The generation of high accuracy fields for comparatively sparse grid arrays is described. Fields are calculated for a range of ion trap geometries which include quadrupole, hexapole and octopole fields (for both rod- and hyperbolic- electrode geometries). Further fields are calculated for quadrupole fields with added higher order multipole components such as hexapole and octopole, generated by altering the geometry of rod-electrode quadrupoles[3]. The multipole components of these fields are analysed. The fields calculated using the high accuracy FDM field solver are used in combination with ion optical ray tracing software to simulate ion trapping within these devices. Simulations of non-linear resonances[4] are described, and modelling of the first stability region of the Mathieu stability diagram[5] is described for several geometries. Non-linear resonance lines are clearly observable in the stability diagrams for several geometries.

The combination of a high accuracy FDM field solver with ion optical ray tracing software gives new insight into non-linear resonances in ion trap devices, and can be used to inform fine details of instrument design.

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PTu-130
13:30 – 14:40

More than one order of magnitude higher sensitivities with Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOFMS)

Eugen Hartungen[2], Alfons Jordan[2], Achim Edtbauer[2], Akio Shimono[1], Simone Jürschik[2], Philipp Sulzer[2], Lukas Märk[2], Tilmann D Märk[2,3]

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Keywords:
PTR-MS, proton transfer reaction mass spectrometry, time-of-flight, PTR-TOFMS, high sensitivity

Novel aspects:
The sensitivity and the limit of detection of two PTR-TOFMS instruments are improved by more than a factor of 10.

Abstract:
In 2009 we published a paper on the development of a high resolution and high sensitivity Proton-Transfer-Reaction Time-Of-Flight Mass Spectrometry (PTR-TOFMS) instrument. Although the coupling of a PTR source to a TOF mass analyzer had already been reported by other groups at that time, our development had an outstanding impact in the PTR-MS community as for the first time a mass resolution of about 7000 m/Δm and a sensitivity of up to 25 cps/ppbv was achieved. Here we report on a number of instrumental developments, leading to serious improvements in sensitivity and in the detection limit, while keeping the mass resolution outstandingly high. We present measurements on gas standards illustrating the advances in instrumental development.
A typical PTR-TOFMS instrument consists of an ion source, where water vapor is converted into H_3O^+ in a hollow cathode discharge and an adjacent drift tube, where the actual proton transfer to the trace gas compounds takes place. The protonated product ions are finally analyzed and detected in a TOF mass spectrometer. It is obvious that for such an instrument a virtually countless number of parameters contribute to the overall performance (instrumental design, lens voltages, orifice diameters, differential pressures, etc.). We consecutively improved and optimized these parameters, which in sum contribute to an increase in instrumental performance of over one order of magnitude without any decrease in mass resolution.
We utilized two different PTR-TOFMS models for the verification of the instrumental improvements, namely a so-called PTR-TOF 8000 (FWHM mass resolution between 5500 and over 8000 m/Δm; increasing with increasing masses) and a PTR-TOF 2000 (mass resolution between 1600 and 2500 m/Δm). As a gas standard we chose a certified aromatics mixture consisting of benzene, chlorobenzene, dichlorobenzene, ethylbenzene, styrene, toluene, trichlorobenzene, trimethylbenzene and xylene (about 100 ppbv each) in nitrogen. At first we connected purified air to the PTR-TOF 8000 and systematically recorded the background signal in order to subsequently determine the limits of detection. Then we connected the gas standard to the instrument and recorded mass spectra (1 s integration time) for about 1 minute. The calculated sensitivities for the corresponding m/z values start with 170 cps/ppbv for the lowest mass 79 m/z (protonated benzene) and increase constantly with increasing mass numbers to 198 cps/ppbv for 113 m/z (protonated chlorobenzene), 215 cps/ppbv for 121 m/z (protonated trimethylbenzene) and 276 cps/ppbv for 147 m/z (protonated dichlorobenzene). Compared to the initial PTR-TOFMS data (published by us in 2009), which ranged from 13 to 26 cps/ppbv, this represents an increase of more than one order of magnitude.
The limits of detection were calculated using the 3 σ method (with σ being the standard deviation of the background signal) for 1 min integration time. The results are 3 pptv for mass 79 m/z down to 1 pptv for 147 m/z.
Currently we are performing similar test measurements on an improved PTR-TOF 2000 which is known to have a higher sensitivity at the cost of a somewhat lower mass resolution. First preliminary data indicate a sensitivity at the high mass compounds of up to 800 cps/ppbv and a detection limit of several hundred ppqv (parts-per-quadrillion). These data will also be presented.

PTu-131
11:10 – 12:20

A curtain-gas filter that widely protects a mass spectrometer from neutral molecule contaminations

Hermann Wollnik, Gary Eiceman, Alexander Tarassov, Ryan Blase, Stephen Devila

New Mexico State University, Las Cruces, USA

Keywords:
ambient air ion sources, elimination of phosphates or silica.

Novel aspects:
Operation of a mass spectrometer with an ambient pressure ion source over extended periods analyzing molecules dissolved in buffers that can cause deposits of nonvolatile contaminations

Abstract:

Introduction

A newly developed curtain-gas filter is presented that operates between the inlet to an evacuated mass spectrometer and an ambient pressure ion source like for instance an electrospray ion source. This curtain-gas filter bars the ion-source buffer gas and all contained neutral molecules from entering the mass spectrometer while it efficiently extracts the ionized molecules from this buffer gas and transmits them into a mass spectrometer.

Action of the curtain-gas filter

In a curtain-gas filter the flow of an ion-source buffer gas streams widely turbulant free for a certain distance parallel to a purified clean gas, while an electric field, that is superimposed over both gas flows, moves ions from the ion-source buffer gas into the purified clean gas. Exclusively this clean gas, that now contains ions, is then entered into the mass spectrometer, while the ion-source buffer gas with all its contaminants is moved to an exhaust. In detail the two gas flows are arranged to both be annular and concentric to the capillary inlet of a mass spectrometer, while the electric field is radial to both gas flows.

First measurements

The curtain-gas filter allowed us to operate a Shimadzu 2020 LC/MS quadrupole mass spectrometer with an electrospray ion source for 100 hours without clogging the capillary inlet or any other part of this mass spectrometer, though the electrospray ion source was fed with a solvent that contained 10mM phosphate. Also after the mentioned 100 hour of continuous flow of phosphate solutions into the curtain-gas filter, there was no perceptible loss in signal of the 1 ppm papaverine sample. Deposits were formed in the easily cleanable ion source container, but not in the inlet to the mass spectrometer or the spectrometer itself. Additionally to the elimination of deposits, cluster ions of low masses and high mobilities, often abundantly available in the plume of an electrospray ion source, are virtually eliminated when the magnitude of the electric field placed over the mentioned two gas flows is chosen to be high enough that the undesired ions of very high mobility are pushed through the flow of the purified clean gas so that they impinge on the conductive inlet capillary. Besides the experiments with phosphates we also used solutions of 1000ppm dissolved silica as used in some cases in flow injection mass spectrometric studies of pharmaceuticals.

PTu-132 High Mass Microscope-mode MALDI Imaging Mass Spectrometry

13:30 – 14:40

Yuto Yanagihara[1], Masahiro Hayashi[1], Yasuhide Naito[2]

[1]Hammatsu Photonics K.K., Iwata,Japan, [2]GPI,Hamamatsu,Japan

Keywords:
MALDI-Imaging mass spectrometry, microchannel plate, detector,

Novel aspects:
An approach aimed at building the standardization method for detection of high mass proteins for MALDI imaging-ms

Abstract:

Introduction

Matrix-assisted laser desorption / ionization imaging mass spectrometry (MALDI-IMS) developed by Caprioli et al. was originally aimed at high mass analytes such as proteins in tissue sections, but is widely used for rather low mass analytes such as lipids. One of the technical barriers to use MALDI-IMS for high mass applications is ion detector sensitivity, which decreases as mass range increases. Ion detector hardware and methods for high mass MALDI have been provided by Covalx AG (Zurich, Switzerland) and installed in some private companies and few public laboratories. Although some applications of MALDI-IMS have been accomplished by those facilities, high mass MALDI-IMS still requires a breakthrough in detector technologies.

We are developing microscope-mode MALDI-IMS, in which trajectories of ions extracted from a point on a sample surface in the MALDI ion source are converged into a focusing point on a detector surface so that the mass-analyzing ion optics keep the spatial distributions of analytes at the sample plate. Because the ion detector for microscope-mode MALDI-IMS is required to be time/position-sensitive, the detector assembly is based on microchannel plates (MCPs) . This study explored the imaging ability of MCP-based detectors in high mass range. In order to achieve a wide mass range that includes high mass proteins, the ion detector technology which is typically used for MALDI-IMS must overcome two challenges ; saturation of the detector caused by intense signals from low mass ions and a decrease in detection efficiency for more massive ions. Increasing the detectable mass range of MALDI-IMS could enable the detection of proteins crucial to biomedical research and other applications. Our main goal is to improve microscope-mode MALDI-IMS where one can obtain subcellular resolution irrespective of the mass region.

Experimental

An MALDI time of flight mass spectrometer, built in-house, was used to examine essential components of microscope-mode MALDI-IMS. The detector consisted of MCPs and a position sensitive anode, which was able to acquire both the positions and arrival times of detected ions simultaneously. The design of the detection system was optimized to enhance the detection efficiency of the MCP-based ion detector so that images of protein distributions could be captured from a model sample.

First, we examined the detection efficiency of MCP-based ion detectors for peptide and protein ions ranging from 1046 to 66430 Da by using our in-house MALDI linear time-of-flight mass spectrometer. We acquired comparison data between masses and signal intensities of the ions.

Second, stigmatic ion images were generated from each spot of peptide and protein samples which were artificially patterned by 400 lines/in. fine grid on the sample surfaces.

Results and Discussion

It was evident that the signal intensity obtained from higher mass ions was much smaller than that obtained from lower mass ions. The gain of electron cascade in MCPs was an important factor in the detection of ions exceeding 10000 Da.

Using a high speed circuit which controlled an MCPs/phosphor screen detector to acquire mass-specific stigmatic ion images while recording TOF spectra, the mesh-patterned ion images were successfully obtained from ions up to 10000 Da. The signal-to-noise ratio of the stigmatic ion images decreased as the mass increased. When the mass of the proteins exceeds 5000 Da, the laser power had to be increased to generate stigmatic ion images.

PTu-133 Analysis of neurosteroids by GC-APPI-MS/MS

11:10 – 12:20

Tina J Suominen, Markus Haapala, Anna Takala, Raimo A Ketola, Risto Kostiainen

Helsinki University, Helsinki, Finland

Keywords:
Neurosteroids, athmospheric pressure photoionization, tandem mass spectrometry, gas chromatography

Novel aspects:
A sensitive GC-APPI-MS/MS method for the analysis of neurosteroids was developed. The method provides efficient separation and ionization of neurosteroids, and an easy way to couple GC to API-MS.

Abstract:
Analysis of neurosteroids by GC-APPI-MS/MS

Tina Suominen[1], Markus Haapala[1], Anna Takala[1], Raimo A. Ketola[2], Risto Kostiainen[1]
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Neurosteroids are generally classified as steroids with local function in the brain, which are formed either in the brain *in situ*, or in the peripheral nervous system regardless of the gonads and adrenal glands. These neuroactive steroids are important concerning several functions in the brain, e.g. protein synthesis, the activation of genes, and the activity of the brain trough the activation of gamma-aminobutyric acid (GABA) - and N-methyl-D-aspartate (NMDA) receptors.(1) The levels of neurosteroids are often altered in the case of neurodegenerative diseases, for example in Alzheimer's disease and multiple sclerosis the levels of neurosteroids have been shown to decrease in some areas of the brain.(2)(3) The concentrations of neurosteroids in the human brain are often very low (pM-nM) and therefore highly sensitive methods are needed. In this work we present a new method for the analysis of neurosteroids in urine by gas chromatography-atmospheric pressure photoionization-tandem mass spectrometry (GC-APPI-MS/MS)

The urine sample pretreatment consisted of enzymatic and chemical hydrolysis of the glucuronide and sulfate conjugates, respectively, followed by liquid-liquid extraction with diethyl ether, and subsequent trimethylsilyl derivatisation of the analytes with MSTFA/NH_4I/DTE. The GC-APPI-MS/MS analyses were performed using a HP 5890 II gas chromatograph coupled to an Agilent 6410 triple quadrupole using a modified version of our earlier presented microchip APPI method.(4) Two columns (Thermo TR-5 MS, length 25m, i.d. 0.25mm, 5 % phenyl 95% dimethylpolysiloxane, film thickness 0.25µm, and Thermo TR-50MS, length 15m, i.d. 0.25mm, 50% phenyl 50% methyl-polysiloxane, film thickness 0.25µm) were sequentially connected for the separation of the analytes. The samples (3 µl) were injected with 1 min splitless injection. The analytical run was initiated at 190 °C, after which the oven temperature was raised to 240 °C at 12 °C/min, from 240 to 255°C at 1 °C/min, from 255 to 330 °C at 12 °C/min, and the column was kept at 330 °C for 2.4 min. The neurosteroids were ionized by photoionisation using a krypton discharge lamp (10 eV) and chlorobenzene as the dopant.

All the 19 steroids were well separated by the used GC method. The trimethylsilyl derivatives of the steroids produced abundant radical cations, which were selected as the parent ions for selected reaction monitoring. The method showed high sensitivity (LODs 0,002- 1 ng/ml) and the repeatability was acceptable (RSD<20%), showing its potential in neurosteroid analysis.

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PTu-134 Potential of direct probe for drug and pharmaceutical

13:30 – 14:40

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Keywords:
APCI, APPI, DirectProbe, time-of-flight

Novel aspects:
Direct probe can detect a solid sample and TLC spots dilectly without melting and extraction.

Abstract:
Detection and identification for pharmaceuticals or drugs are generally used with chromatography. Compositions are extracted from complicated samples with chromatography technology, and then identification for the composition are performed by means of specified color reaction, specified absorption, mass spectrometry and so on.
Therefore, sample preparation and waste time are disadvantage in order to remove obstructions from complicated samples.
Direct Probe is one of accessary for mass spectrometry. This is one of technology which are useful for APCI (Atmosphere pressure chemical ionization) or APPI (Atmosphere pressure photo ionization) . In generally, soluble sample are treated with LCMS, while Direct Probe can treat with not only soluble sample, also solid sample.
This characteristic is that this measurement is simple and quick detection.
This is a solution which solves problems, such as time waste and procurement of a sample.
Additionally, when there is no telling into what kind of solvent a sample melts, you can directly detect solid sample with this probe.
In this time, we will introduce easy and high accuracy technology which combined this direct probe to a time-of-fright mass spectrometry.
Drugs or these metabolites were added to a physiological saline solution, and these compositions could be detected with this system easily, and then we will discuss limit of quantification for drugs and these metabolites in physiological saline solution with this direct probe.
In the case of such complicated sample as traditional medicine and mixture sample,
We use thin layer chromatography (TLC) as preparation and then we will introduce some data for these TLC spots.

PTu-135
11:10 – 12:20

Development of high-speed data-streaming system for time-of-flight mass spectrometry

Ken-ichi Bajo[1], Osamu Fujioka[2], Shingo Ebata[1], Morio Ishihara[3], Kiichiro Uchino[4], Hisayoshi Yurimoto[1]

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Keywords:
High speed data acqisition, TOF-SNMS, SIMS

Novel aspects:
High speed data acquisition system was developed which is important for imaging. We utilized PXI-platform in order to process a large amount of data of TOF mass spectra.

Abstract:
Secondary ion mass spectrometry (SIMS) is useful to determine elemental and isotopically abundances of various materials in a microscopic region. Multi-isotopes can be simultaneously detected by using time-of-flight mass spectrometry (TOF-MS), which is one of the features for TOF-MS. We are developing a spattered neutral mass spectrometer (Laser Ionization MAss nanoscope : LIMAS [1]). LIMAS is consisted of focused Ga ion beam in order to examine a nanometer-scale region, femtosecond laser which ionized spattered neutral particles, and multi-turn TOF-MS (MULTUM-II, [2]).
Secondary ions of each isotope (or compound) pass through the MULTUM-II. Mass-separated ions are detected by ion detection system with micro channel plate (MCP). High-speed digitizer with an analog bandwidth of >GHz is required because a timescale of the ion detection is a few nanoseconds (ns). Extracted ions by single spatter are about ten thousand less than those by continues beam because pulse width of the primary beam is ~200 ns. The low extraction rate leads directly to a low signal output. A pulse-counting method in common use can dramatically increase a signal-to-noise ratio and obtain signal intensity quantitatively. In an ion detection system of LIMAS we established high speed data acquisition and processing procedure with NI PXIe-platform. Two MCPs and preamp amplify the introduced electrical signal. The input signal is split by a distributer called Wilkinson coupler. The output signal is recorded by using two NI PXIe-5185s (hereafter 5185). One is for recording the analog output, another is for pulse-counting. 5185 digitizer makes it possible to obtain mass spectra in high precision by ultra-fast sampling rate (8-bit vertical resolution and 12.5 GS/s). High data transfer speed (up to 1 GB/s) by using PCI Express bus provides real-time data acquisition and deconvolution and high sampling rate up to 10 kHz.
The wave forms are recorded for several microseconds by post-trigger. In case of pulse-counting, the recorded array is simultaneously transformed to 1/0 one. An algorism of the deconvolution is as follows. When a center value of three arbitrary consecutive points is higher than the other two ones and a threshold which is previously determined from pulse height distribution, the value of the center index is "1". The other cases are "0". A pulse height distribution is fundamental to the pulse-counting because a pulse from the ion has to be separated from noise. The pulse energy and noise level are distributed to 29 ± 28 mV and 2 ± 3 mV in 3-sigma, respectively. The ion pulse can be distinguished from noise.
Short dead-time and data streaming can allow the pulse-counting for TOF-MS. A time resolution of this method depends on the sampling rate of 5185 (currently 3.125 GS/s) and responsively of the MCP assemblage (full width half maximum ~2.5 ns). Consequently, it is about 1 ns which is consistent with the three data points (0.96 ns). In case of constant recurrence timing the system acquires ions from one to 1 × 10^9 cps. Since there is statistical fluctuation for incident ions, the ions are detected up to 1 ×10^8 cps for practical purpose. In case of a high intensity signals (>10^8 cps) the signals can be recorded by another 5185 as ananalog one. A dynamic range of the system is >10^{10} because the analog signals can be input up to 10^{11} cps.

[1] Ebata et al. (2011) SIA, DOI : 10.1002/sia.4857.
[2] Okumura et al. (2005) Eur. J. Mass Spectrom., 11, 261.

PTu-136
13:30 – 14:40

Mass coverage range enhancement of reflectron time-of-flight mass spectrometers by superconducting nano-stripline detectors

Nobuyuki Zen[1], Koji Suzuki[1], Shigetomo Shiki[1], Masahiro Ukibe[1], Masaki Koike[1], Alessandro Casaburi[2], Mikkel Ejrnaes[2], Roberto Cristiano[2], Masataka Ohkubo[1]

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Keywords:
MALDI-TOF MS, reflectron, superconducting nano-stripline detector, SSLD

Novel aspects:
The superconducting nano-stripline detector (SSLD) which has the detection area of 5×5 mm 2 with the fast response time of several ns was realized.

Abstract:

Introduction

Superconducting detectors are promising for next-generation ion detectors for time-of-flight mass spectrometry (TOF MS). They can achieve a mass-independent detection efficiency even for macromolecules, because output pulses are produced through the deposited kinetic energy at ion impact instead of secondary electron mission that is the ion detection mechanism of conventional microchannel plate (MCP) detectors or secondary electron multipliers (SEM). Among the superconducting detectors, superconducting nano-stripline detectors (SSLDs) which consist of several hundreds of superconducting lines with a width of a few hundreds nm and a thickness of a few tens of nm have a fast response time less than 1 ns. We succeeded in realizing the detector size up to 5×5 mm^2 without response time degradation by using parallel nano-stripline configuration. An SSLD installed in a reflectron TOF MS enables significant enhancement of the mass coverage range.

Experiments

Generally speaking, the response time of SSLDs is determined by the kinetic inductance of superconducting striplines, and thus longer striplines result in a slower response time. Our strategy to realize a large detection area (or a long stripline) without degradation of the response time is to connect striplines in a parallel configuration. In this study, we connected 50 striplies in parallel and the detector size of 5×5 mm^2 was realized.

The SSLD was installed in a reflectron TOF MS (Voyager DE-STR ; AB Sciex Pte. Ltd.). The SSLD is cooled down by a cryogen-free cryostat, and the temperature of 3.2 K is easily obtained without liquid helium. The biomolecular samples are ionized by matrix assisted laser desorption/ionization (MALDI) and those ions are accelerated by a high voltage of 25 kV.

Results and Discussions

By measuring Angiotensin I (1,296 Da), ACTH (2,565 Da), Lysozyme (14 kDa), and IgG (146 kDa) with the 5×5 mm^2 SSLD, we have confirmed that the rise and fall time is less than 1 ns and less than 3 ns, respectively, and the pulse shape is independent of molecular ion species.

In addition to the success in the enlargement of the detection area, another important progress is the refinement of the operation condition. Usually, the SSLD is driven by the bias current, and the larger bias current is necessary to achieve 100 % detection efficiency. However, previous SSLDs made of the niobium superconductor were easily latched at the normal state after single ion bombardment, and the bias current was limited at most 60 % of the critical current. In this study, the width of the jointing part of striplines was enlarged up to 10 times wider, which realized the 95 % current biasing without the latching problem.

In order to investigate the performance of our TOF MS, we measured a mixture of Lysozyme and IgG with a sinapic acid matrix. Their mass peaks were recognized and the wide mass coverage range was achieved in the reflectron TOF MS.

PTu-137 An Improvement of MCP Dynamic Range for TOF MS Application

11:10 – 12:20

Yusuke Hayase, Uchiyama Toshiyuki, Ishiguro Katsuyuki

Hamamatsu Photonics, Iwata, Japan

Keywords:

MCP, Dynamic Range, Linearity, Ion Detector, Microchannel Plate

Novel aspects:

We have found the best material composition and formation to strike a balance between wide dynamic range and robustness. Then we have developed the robust and wider dynamic range MCP.

Abstract:

Microchannel Plates (MCP) are widely used for Time-Of-Flight Mass Spectrometry (TOF MS) because they have a fast time response less than 1 ns, small time jitter, long life time, and a large effective area, which are desirable features for TOF MS. However, the electron multiplier tube (EMT) is still used in MS applications that require a wider dynamic range since the MCP's dynamic range is limited. Another limitation regarding MCPs is their propensity to warp and crack easily when stored for long periods of time, which makes the time jitter worse. In order to solve this big problem, we have already developed a "Robust " MCP which will not warp and crack as easily during these long storage periods. Now, we have developed a "Robust " MCP with a wider dynamic range that will output as much current as EMT.

Dynamic range is dependent on the MCP strip current, which supplies electrons to the MCP channels. When there is a large incoming ion signal, electrons are not supplied fast enough from the power supply to match the increase in output current. In order to solve this problem, an MCP with a lower resistance is required. Since the MCP's resistance is related to its material composition, we researched many different materials and found the best material composition and formation to strike a balance between wide dynamic range and robustness.

We evaluated the dynamic range of an MCP, 20mm dia. active area, 12um channel dia. and 0.48mm thickness, made from the above material composition and formation. We confirmed the dynamic range of the MCP extends to 35uA, 5 times wider than the original MCP, and expect the future versions will extend to 100uA. That means the developed MCP has the equivalent output dynamic range to EMT. In terms of robustness, we did an accelerated test under 40 degree Celsius and 93% humidity environment during 16 days, which corresponded to 1000 days storage under a regular environment. Flatness of a normal, non-robust, MCP was over 150um or cracked after the storage, but flatness of the robust MCP did not change.

We will employ this technology in larger MCPs like 25mm or 40mm active area, a smaller channel MCP like 6 um and a thinner MCP like 0.3mm.

The poster will show detailed material composition, formation and evaluation results.

PTu-138
13:30 – 14:40

Specific analyte ionization using negative atmospheric ions formed in atmospheric pressure corona discharges

Kanako Sekimoto, Mitsuo Takayama

Yokohama City University, Yokohama, Japan

Keywords:
Corona discharge, Negative atmospheric ion

Novel aspects:
It has been found that various atmospheric negative ions formed in atmospheric pressure negative corona discharges can be useful for specific analyte ionization.

Abstract:
APCI-like ambient ionization techniques such as DART and ASAP have frequently employed corona discharge devices which lead to abundant generation of the atmospheric ions utilized as reactant ions for analyte ionization. These techniques, however, have some problems especially in *negative*-ion mode, e.g., the complexity of negative-ion mass spectra consisting of not only deprotonated analyte peaks but also a number of peaks of molecular attached and/or fragment ions and atmospheric negative ions, some of which could not be identified. In order to accurately interpret the negative-ion mass spectra obtained, it is necessary to understand the influence of atmospheric negative ions on analyte ionization. Here we regulated the formation of atmospheric negative ions such as HO^-, CO_x^- and NO_x^- in ambient corona discharges, and systematically investigated the interaction between individual atmospheric ions and various type organic compounds.
The corona discharge ion source used contained a needle, whose tip surface was adequately approximated by a hyperboloid of revolution, and metallic orifice plate of a TSQ7000 triple-quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Discharge ionization was performed under atmospheric pressure in laboratory air with relative humidity of 50-70 % at 298 K.
The formation of atmospheric negative ions in ambient corona discharges was regulated by the electric field strength on the needle tip surface, which is determined by combination of the gap between the point-to-plane electrodes, the DC voltage applied on the needle, and the local curvature of the needle tip surface. The dominant atmospheric ion formed at the low field strength below 10^7 Vm^{-1} was the superoxide anion O_2^-. At medium field strength (10^7-10^8 Vm^{-1}), the negative ions originated from nitrogen dioxide NO_2, oxalic acid $(COOH)_2$ and carbonic acid H_2CO_3, i.e., NO_2^-, $COO^-(COOH)$ and HCO_3^-, were generated. The high field strength above 10^8 Vm^{-1} led to the formation of abundant NO_3^- ions and its complexes with HNO_3, i.e., $NO_3^-(HNO_3)_n$ (n = 1,2).
The relationship between the field strength and the resulting atmospheric negative ions could be interpreted from the standpoint of the field-emitted electron kinetic energy which is the principle factor governing the sequential progress of successive ion-molecule reactions in the formation of individual atmospheric ions.
Atmospheric negative ions described above could serve as reactant ions R^- for analyte ionization. The individual atmospheric ions R^- *specifically* ionized analyte molecules *M*, consisting of aliphatic and aromatic compounds with several functional groups such as methanol, 2-phenylethanol, acetic acid, benzoic acid, phenol, phenylethylamine, 4-aminobutanoic acid, and amino acids, to atmospheric ion adducts $[M+R]^-$. The adducts of R^- with higher proton affinities, i.e., O_2^-, HCO_3^- and NO_2^-, furthermore, resulted in the production of deprotonated analytes $[M-H]^-$ due to the efficient proton abstraction from *M* by R^-. The abundances of the individual atmospheric ion adducts $[M+R]^-$ and resulting $[M-H]^-$ formed under certain field strength conditions were in good agreement with the concentration of R^- formed under those conditions. The results obtained suggested that the formation of $[M+R]^-$ and $[M-H]^-$ under certain discharge conditions is attributed to the chemical affinity between *M* and R^- and the concentration of R^- produced under these conditions. The CID experiments of all the atmospheric ion adducts $[M+R]^-$, furthermore, showed the characteristic fragmentation behavior for the individual atmospheric ions R^-, in which deprotonated analytes $[M-H]^-$ and/or the atmospheric ion R^- were mainly formed depending on the proton affinities of $[M-H]^-$ and R^-. The CID data obtained will be useful to determine the molecular mass of unknown compounds measured by ambient ionization techniques using corona discharges.

PTu-139
11:10 – 12:20

Probe Electrospray and Nanoelectrospray Ionization for Direct Biomolecule Analysis and Cancer Diagnostics

Mridul Kanti Mandal[1], Lee Chuin Chen[2], Kentaro Yoshimura[3], Subhrakanti Saha[1], Obaidur Rahman[1], Matiur Rahman[1], Yasuo Shida[1], Satoshi Ninomiya[2], Sen Takeda[3], Kenzo Hiraoka[1]

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Keywords:
Ambient Mass Spectrometry, Cancer Diagnostics

Novel aspects:
The novel characteristics of PESI-MS can fulfill the requirements of clinical mass spectrometry based cancer diagnostics. Solid Probe assisted nanoESI can also be used as a cancer diagnostic tool.

Abstract:
[PESI-MS] : Biological samples normally contain high concentration of salts which are needed to remove by purification methods prior to successful omics analysis with mass spectrometry. In addition, detergents are necessarily used for extraction protocols of membrane proteins from biological cells or tissues. After extraction, elimination of detergent is indispensable for the better performance of ESI-MS which is usually tedious and time consuming.

In contrast, PESI-MS developed in our laboratory has been successfully applied to the sample solutions that contain high-concentration salts and detergents with no special sample pretreatment. The mass spectra of PESI were measured as a function of time for a mixture of several analytes. Intriguingly it was found that the analytes were electrosprayed sequentially in the order of their surface activities during the period of electrospray. To fulfill the clinical goal and optimize the conditions for molecular diagnosis of malignant tumors, PESI-MS has also been adopted for human pathology. It is found that PESI-MS has successfully delineated the differential expression of phospholipids (PCs) and triacylglycerols (TAGs) in non cancerous and cancerous kidney tissues. Therefore, PESI-MS can be used as a diagnostic tool during surgery or after biopsy.

[Solid probe assisted nanoESI] : Extraction procedures are mandatory for successful nanoelectrospray ionization (nanoESI) of biomolecules from tissue specimen. To eliminate these procedures, solid probe assisted nanoESI has been newly developed. This technique could minimize the conventional extraction protocols for analyzing biological tissues and cells.

In this method, 0.3 mm acupuncture needle was used for sample loading from biological tissue directly. The needle was simply stuck to the biological tissue. After sampling, the needle was inserted from the backside of nanoESI capillary in which 1 μL solvent was loaded in advance. By the application of high voltage, the extracted samples in solution were nano-electrosprayed. This method is shown to be simple and easy to operate. By this method, diseased and healthy tissues could be clearly discriminated. Since the direct sampling by acupuncture needle is comparatively much less destructive than conventional hypodermic needle-based biopsies, the solid probe assisted nanoESI would be a useful tool for biological tissue diagnostics with lowest invasiveness.

References:

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[2] M. K. Mandal, L. C. Chen, Z. Yu and K. Hiraoka. J. Mass Spectrom. 46 (2011) 967975.

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PTu-140
13:30 – 14:40

Development of ultra high sensitive ambient ionization mass spectrometry using Leidenfrost phenomenon-assisted thermal desorption

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Keywords:
Ambient MS, Leidenfrost phenomenon

Novel aspects:
The application of Leidenfrost phenomenon to the ultra high sensitive ambient ionization mass spectrometry was made. A variety of compounds could be detected as molecular ions.

Abstract:
In ambient sampling/ ionization mass spectrometry, desorption of analytes plays a significant role for the high-sensitive detection of less- or non-volatile compounds. Present work demonstrates a new sample desorption technique based on Leidenfrost phenomenon. When a liquid droplet is dropped on a high temperature (temperature much higher than the solvent's boiling point) solid metallic surface, the liquid immediately vaporizes on the solid surface and the vaporized gas layer prevents the droplet to contact with the metallic surface, i.e., the droplet levitates above the surface. This phenomenon is known as 'Leidenfrost phenomenon' and the temperature above which this phenomenon occurs is called as 'Leidenfrost temperature'. During this process liquid droplet evaporates rather slowly due to the presence of the heat-insulating vapour layer under the droplet. Thus, the temperature of the droplet is kept below the solvent's boiling point. Thus, the non-volatile analytes in solution do not evaporate but are retained in solution until the last moment of the total liquid evaporation. At the last moment of droplet disappearance, desorption of non-volatile compounds takes place accompanied by explosive solvent evaporation. The evaporated/desorbed analytes were ionized by a dielectric barrier discharge (DBD) ion-source. This method was applied to illicit and therapeutic drugs, explosives, toxicants, steroids, peptides, etc. and was found to be applicable to all of these compounds irrespective of their volatilities. The lower limits of detection ranged as low as parts per trillion (PPT) level for drugs, toxicants and steroids (sample amount fg to pg level) , parts per billion (PPB) level for explosives and 10^{-9}M for some peptides (e.g., Cyclosporin A) . The high sensitivity of the present method is due to the spontaneous enrichment of non-volatile analytes during the slow and smooth evaporation of the solvent. As the temperature of the droplet does not rise above the boiling point of the solvent, molecular ions are observed as the major ions. This newly developed desorption technique is simple, rapid and high-throughput.

PTu-141 Scanning Probe Electrospray Ionization for Ambient Mass Spectrometry

11:10 – 12:20

Yoichi Otsuka[1], Sayuri Shide[2], Junpei Naito[1], Masafumi Kyogaku[1], Hiroyuki Hashimoto[1], Ryuichi Arakawa[2]

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Keywords:
Ambient sampling ionization, Tissue imaging

Novel aspects:
New atmospheric-pressure sampling / ionization method by single capillary probe. The liquid bridge and electrospray at the side of the probe makes it possible to map the biological components.

Abstract:
Introduction
Imaging mass spectrometry (IMS) with atmospheric-pressure ionization method has received widespread attention because of the possibility of rapid diagnosis. Desorption electrospray ionization (DESI) was developed in 2004, and it has received strong attention, so far. DESI utilizes charged solvents and high-pressure nebulizing gas for the ionization process. Recently, nano-DESI was developed which utilizes charged solvents and two capillaries aligned in a way to separate the desorption and ionization steps. This method has two advantages over DESI such as improved detection limits and higher spatial resolution. However, the alignment of capillaries is also needed. We have developed the atmospheric-pressure ionization method named "Scanning Probe Electrospray Ionization (SPESI) " which uses a single capillary as the probe for ionization.
Methods
SPESI utilizes the capillary and charged solvents like DESI and nano-DESI, but this method have the advantages of simplicity compared to these methods ; High-pressure gas as well as the alignment of two capillaries are unnecessary. SPESI enables the local desorption and ionization by using a liquid bridge and electrospray with single probe. A charged solvent is flowing through the probe. As the probe and the sample are close-positioned, a liquid bridge is formed between them. Immediately after the components are dissolved by the liquid, they are ionized by electrospray ionization.
Preliminary data
The formation of the liquid bridge and the movement of the probe are observed by high-speed camera. We have found two different operating modes in SPESI. One is named as "Contact Mode SPESI " in which the position of the probe is kept close to the sample surface. In this mode, the liquid bridge and Taylor cone are formed simultaneously. The other is named as "Tapping Mode SPESI " in which the probe is vibrating so as to separate the formation of liquid bridge and electrospray ionization. We found that the control of the fluid motion of charged solvents from liquid bridge to Taylor cone was the key to operate these modes stably. One dimensional mapping of solid sample by SPESI was also studied. The position of sample was controlled by the single-axis moving stage. Each of reference samples and tissue sample were ionized by these two methods. As reference sample, insulin (5.8kDa) and bovine serum albumin (BSA, 66kDa) solution was deposited on the PTFE substrate to make small spots. Ionization along several spots was performed by contact mode SPESI. It was confirmed that the distribution of ion intensity along the moving direction was corresponding to the position of the spots. Moreover, the formation of multivalent ions shows SPESI is a soft-ionization method. Tissue samples were studied subsequently. Normal mouse pancreas was cryosectioned and mounted on the glass substrate. It was dried under atmosphere and used without further treatment. Ionization along several thin tissues was performed by tapping mode SPESI. The result indicated that various kinds of components were ionized on the expected sites of the tissue samples. Our preliminary results indicate that SPESI can be used for the ambient sampling and soft ionization of biomaterials. Moreover, the one-dimensional mapping of both BSA film and mouse tissue shows that SPESI can be used for imaging mass spectrometry.

PTu-142
13:30 – 14:40

Simultaneous Ionization of Nonpolar and Polar Compounds by Laser Ablation Electrospray Ionization with Heated Gas Jet

Anu Vaikkinen[1], Bindesh Shrestha[2], Akos Vertes[2], Risto Kostiainen[1], Tiina J Kauppila[1]

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Keywords:
ambient ionization, laser ablation, electrospray, nonpolar, apolar

Novel aspects:
Simultaneous analysis of polar, nonpolar and low polarity compounds by heat-assisted laser ablation electrospray ionization-mass spectrometry

Abstract:
Laser ablation electrospray ionization (LAESI) is an ambient mass spectrometric ionization method that can be used for molecular imaging, and the analysis of small cell populations and even single cells. In LAESI, a mid-infrared laser is used to ablate water-containing samples producing a plume of vapor particulate projectiles. The ablated material is ionized by electrospray, after which the ions are guided into a mass spectrometer for analysis. The ablation is highly localized with ablation areas in the range of 30 to 300 microns in diameter. LAESI efficiently ionizes polar compounds such as proteins, peptides and phospholipids, while the ionization of nonpolar compounds is often less efficient. In this work we show that by assisting the LAESI process by heated gas flow the ionization of low polarity compounds such as steroids, and even completely apolar compounds, such as polyaromatic hydrocarbons (PAHs) can be accomplished.

As in conventional LAESI, the novel set-up used a mid-IR laser running at 2940 nm to ablate analytes from water-containing samples. Analyte ionization was induced by an orthogonal electrospray of water/methanol (1 : 1, v : v) + 0.1 % HCOOH. The spray angle was approx. 50-60° respective to the mass spectrometer inlet. To facilitate the ionization of low polarity compounds, the electrospray plume was intercepted by a heated nitrogen jet (~200 degrees Celsius) that was generated using a heated microchip positioned on-axis to point directly toward the inlet of the mass spectrometer.

The novel heat-assisted LAESI ion source was found to enable the simultaneous ionization of polar and low polarity compounds in a single measurement. As expected for LAESI, polar compounds such as bradykinin fragment 1-8 were efficiently ionized without the heated nitrogen flow. Low polarity compounds, such as estrone, were ionized only when the heated gas jet was applied. The gas flow rate did not have a significant effect on the peak intensity of the bradykinin fragment 1-8, whereas the signal of estrone increased 19-fold when the gas flow rate was raised from 30 to 360 mL/min.

The heat-assisted LAESI method was used to analyze 10 microliter aliquots of 100 micromolar standards in water/methanol (1 : 1,v : v) placed on a glass microscope slide. Protonated molecules were observed for, e. g., naphtho [2,3-a] pyrene, dehydroepiandrosterone, estrone, verapamil, and 1,2-dioleoyl-sn-glycero-3-phosphocholine, while bradykinin fragment 1-8 showed MH^+ as well as $[M+2H]^{2+}$ ions in the spectrum. Thus the ionization of the tested compounds was somewhat similar to electrospray ionization, except that apolar compounds with low proton affinities, such as naphtho [2,3-a] pyrene and dehydroepiandrosterone were also detected. Some compounds produced structure-specific fragments, e. g., cholesterol showed $[M\text{-}OH]^+$ and 1-palmitoyl-2-oleoyl-sn-glycerol $[M\text{-}OH]^+$ and ions formed by loss of single fatty acyl chain.

The sensitivity of the method was explored by determining the limits of detection for bradykinin fragment 1-8 and estrone. Volumes of 0.5 microliters in water/methanol were analyzed on a cooled Peltier stage to avoid rapid sample evaporation. Bradykinin fragment 1-8 and estrone were detected from 2.5 and 6 micromolar solutions, respectively, giving absolute limits of detection of 1.3 pmol for bradykinin fragment and 3 pmol for estrone. The LODs were somewhat similar to those obtained with previously established techniques, as laser ablation atmospheric pressure photoionization gave LOD of 3 pmol for estrone and LAESI without the heated gas jet detected 0.65 pmol of bradykinin fragment 1-8. The novel heat-assisted LAESI method produced linear response over 3 decades for the signal of verapamil with R^2 of 0.99 ($n = 11$).

The novel technique was applied in lipid profiling of plant (avocado fruit) and animal tissues (mouse brain). The main lipid species observed from the brain was cholesterol, whereas avocado showed abundant triglycerides.

PTu-143
11:10 – 12:20

Desorption atmospheric pressure photoionization-mass spectrometry for direct analysis of tetrahydrocannabinol from Cannabis sativa samples

Tiina J Kauppila[1], Anu Flink[1], Ulla-Maija Laakkonen[2], Laura Aalberg[2], Raimo A Ketola[1]

[1]University of Helsinki, Helsinki, Finland, [2]National Bureau of Investigation, Vantaa, Finland

Keywords:
ambient ionization, desorption atmospheric pressure photoionization, drug analysis, Cannabis sativa, molecular ion

Novel aspects:
DAPPI can be used for the rapid and reliable analysis of THC from Cannabis sativa samples due to the specific CID fragmentation pattern of the molecular ions formed in DAPPI

Abstract:
The recent innovation of ambient mass spectrometry techniques has speeded up the analysis of confiscated drugs in forensic laboratories. These techniques make possible the fast and direct analysis of confiscated drugs in their solid forms, such as tablet, resin or plant, completely without sample preparation or chromatography. However, a serious problem is related to the analysis of *Cannabis sativa* originating drugs : most ambient analysis techniques cannot differentiate between the active ingredient tetrahydrocannabinol (THC) and a non-active ingredient, cannabidiol (CBD) , which have the same molecular masses. This is because most ambient techniques rely on the formation of protonated molecules of THC and CBD that have similar CID fragmentation patterns. However, desorption atmospheric pressure photoionization (DAPPI) can be used to form molecular ions that - similarly to the molecular ions formed in EI - have individual fragmentation patterns. Here, we show that this feature of DAPPI makes possible the fast and specific analysis of THC from *Cannabis sativa* samples.

The dried *Cannabis sativa* samples were placed on a moving stage outside the mass spectrometer and analyzed as such, using a home-made DAPPI source. Hot solvent vapor and nebulizer gas were delivered towards the sample using a heated nebulizer microchip, causing the thermal desorption of the analytes from the sample surface. Nitrogen was used as the nebulizer gas and toluene as the spray solvent. A krypton discharge VUV lamp with 10.0 and 10.6 eV photon energy was used to initiate the ionizing reactions.

A set of 10 *Cannabis sativa* samples with different, known proportions of THC and CBD was chosen. The DAPPI mass spectra of all the samples showed intense ions at m/z 314, which can be the $M^{+\cdot}$ of either THC or CBD, formed by charge transfer. The product ion spectra of m/z 314 were collected and compared visually, and in addition, principal component analysis (PCA) of the MS/MS spectra was performed using Unscrambler X (Version10.0.1, CAMO Software, Oslo, Norway) . The visual observation of the product ion spectra showed that the spectra of the samples containing mainly THC were significantly different from those containing mainly CBD. The product ion spectrum of the $M^{+\cdot}$ of THC showed the most intensive product ions at m/z 299, 243, 231, 258, 272, 313, and 221 (in the order of decreasing signal) , while the product ion spectrum of CBD showed the most intensive product ions at m/z 272, 195, 258, 299, 108, 231, 208, 207, 206, 120, and 243. It would thus be possible for a forensic laboratory to visually differentiate the samples containing high amounts of THC from the samples containing no THC. However, to test whether it would also be possible to differentiate the samples containing small amounts of THC from those containing none, a PCA analysis using the relative intensities of the MS/MS product ions as variables was performed. To optimize the PCA components in order to find the simplest possible model, several combinations of ions were explored. The most simple model was found by choosing the relative intensities of just two variables : the product ions at m/z 272 and 299. This model gave an explained variance of 100 % and made possible the differentiation of *Cannabis sativa* samples with high, intermediate and low contents of THC from those that only contained CBD. The differentiation of THC and CBD containing *Cannabis sativa* samples is thus possible with DAPPI by using the developed PCA model as the reference or just by visually comparing the spectra and the proportions of product ions at m/z 299 and 272 in the product ion spectrum of m/z 314.

PTu-144
13:30 – 14:40

Open Spot Ambient Ionization Technology for Near-Instant Determination of Sample Characteristics by using DART-MS

Musselman D Brian, Joseph LaPointe, Elizabeth Crawford

IonSense, Inc.

Keywords:
DART, Ambient Ionization, Chemical Identification

Novel aspects:
Rapid ionization with electrical current-based thermal desorption facilitates near-instant ionization

Abstract:
A new direct analysis in real time (DART) source has been developed to facilitate rapid desorption chemical ionization of samples from wire mesh, OpenSpot Cards, designed to carry optimium volumes of sample. The use of the wire mesh coupled to a high current power supply facilitates a simplified approach to the sampling process associated used in qualitative mass spectrometry with DART-MS. OpenSpot cards are ideal for loading either solid or liquid samples since it is only necessary to scrape the solid across the screen surface or dispense a microliter of sample laden solvent in the sample region to facilitate analysis. The simplicity of using this thermal desorption ionization method facilitates Transmission mode DART ionization. Application of the technology to rapid identification of products of organic synthesis facilitating with high resolution-MS will highlight the utility of the method for reaction monitoring. Detection of contaminants in products with low resolution compact MS and Principle Component Analysis will be described as a means to improve quality control while reducing the cost per analysis. Finally, qualitative and quantitative analysis of pesticides and environmental contaminants in various commercial products by using MS/MS will be featured with emphasis on the use of the method to facilitate screening in advance of full analytical determinations.

PTu-145
11:10 – 12:20

Plasma pencil atmospheric mass spectrometry for the detection and measurement of micronutrients in complex solutions

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Keywords:
ambient ionization, nutrient measurement

Novel aspects:
This work uses ambient plasma ionization for the separation of analytes in complex solutions based on concentration, and applies a Bayesian regression model for analyte quantitation.

Abstract:
Malnutrition, particularly in the form of micronutrient deficiencies, is a major contributor to the global burden of disease. Insufficiencies in any of the key micronutrients, specifically vitamin A, iron, zinc, folic acid and iodine, can cause critical health problems. Pregnant women and children are particularly vulnerable. While present in all countries, malnutrition is of higher concern in less developed countries. This is due in part to a lack in established food fortification procedures. Additional nutritional epidemiology of these groups would be beneficial to determine which nutrients and quantities are necessary to achieve optimum health in these regions ; however, the high cost and time consuming nature of current micronutrient measurement methods make large studies unfeasible. Our goal is to utilize a relatively new technique, plasma pencil atmospheric mass spectrometry (PPAMS) , as a field-deployable method to simultaneously measure the five micronutrients in blood. A multivariate software model is then used to quantitatively assess the resulting fragment spectrum.

The PPAMS system was assembled in house by constructing a low temperature plasma probe and coupling it to a Bruker Esquire LC mass spectrometer (Bruker Daltronics, Billerica, MA) . Sample spectra were collected in the m/z range from 50-1100 m/z, after ionization with a helium based plasma. Sample matrices were mixed using three physiologically-relevant blood levels (BLC, healthy, average and critically low) of each of the five micronutrients, doped into a 10% porcine plasma solution. Samples were analyzed to determine the sensitivity of our device and the ability of the multivariate technique to separate the nutrient populations based on concentration. Initial studies have focused on varying three nutrients (iron, zinc and vitamin A) at each of the three levels while holding iodine and folic acid at the critically low BLC. Future studies will be expanded to include all nutrients. Samples were kept in liquid form and analyzed directly.

Data analysis using principal component analysis (PCA) of the positive ion spectra was completed by grouping data sets to vary a single nutrient while holding the other nutrient levels constant. The resulting scores plots show clear separation at the 95 % confidence level between low, medium and high levels of each nutrient, with separation occurring proportionally to concentration. From this separation, we can study the loadings plots to determine the specific peaks associated with individual nutrients.

Quantitation is being completed through the use of a Bayesian regression model. Initial samples at high, medium and low BLCs are being used as a test matrix to form the model, which will be used to analyze additional samples. In this method, discrete m/z data points are converted into a wavelet function to model the system, which is then applied towards "unknown " spectra to estimate the nutrient levels in the unknown samples. Future work will combine the two systems to improve data prediction by focusing on specific peaks that are related to nutrient concentration.

Acknowledgements : We thank the Bill and Melinda Gates Foundation for generous funding. The authors would also like to acknowledge Drs. Martin Sadilek, Chris Barnes and Marvi Matos for their shared expertise and assistance.

PTu-146
13:30 – 14:40

Rapid screening and quantitation of pesticides in fruit commodities by Direct Analysis in Real Time (DART) ambient ionization mass spectrometry

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Keywords:
Pesticide Screening, Direct Analysis in Real Time (DART), Multiple Reaction Monitoring (MRM), High Resolution Accurate Mass MS/MS

Novel aspects:
Porous sampling surfaces and automation allow for rapid screening and quantitative analyses of pesticides in juices by DART-MS.

Abstract:

INTRODUCTION

Routine pesticide and fungicide use in the United States, as well as abroad warrants the need for analytical techniques that can rapidly screen and quantify residues in order to efficiently sample products before reaching the consumer market. The allowable residue levels of pesticides on fruits and vegetables that are processed into juices are governed in the US by the United States Environmental Protection Agency (US EPA) Code of Federal Regulations Title 40 : Part 180 that sets tolerance levels based on the produce commodity. Of particular interest in the United States, carbendazim was found in imported orange juice from Brazil, where the use of the fungicide is legal. The US does not permit the use of carbendazim on any citrus fruits and therefore any imported citrus juice is held to the same regulation. Ambient ionization offers the ability to screen fruit juice samples directly in seconds and with automated sample introduction quantitative measurements can be assessed using direct analysis in real time (DART®) mass spectrometry.

EXPERIMENTAL

A fourth generation standardized voltage and pressure DART ionization source (DART-SVP) was fitted with a motorized linear rail and transmission experiment module. In transmission DART experiments the liquid juices are suspended onto a stainless steel mesh and the DART ionizing gas passes through the porous sample support efficiently ionizing the analytes. A number of juices (cranberry, grape, orange, lemon and a vegetable mix) were spiked with the following pesticides : diazinon, carbaryl, metalaxyl, difenoconazole, pyridaben, dimethomorph, azoxystrobin, fenhexamid, pyriproxyfen and spiroxamine. The pesticide mix was spiked at levels ranging from 10 ppb to 5 ppm with caffeine added as an internal standard at 100 ppb. Carbendazim was also spiked into orange juice at 1 ppb to 5 ppm and orange juices from India (4), Europe (5) and USA (3) were screened for carbendazim. The DART source was interfaced with a QTRAP and quadrupole Orbitrap benchtop instruments.

RESULTS

An API 4000 QTRAP was tuned for MRM conditions for the 10 pesticides in the spiking mixture as well as carbendazim using the TurboV source with ESI probe. The spiked fruit juices were spotted (3 µL spots) onto a stainless steel mesh and the spots were allowed to dry before analysis. The samples were driven through the DART ionization region using the motorized linear rail at a speed of 0.5 mm/s resulting in a single sample time of 25 seconds. A preliminary set of runs were conducted on the solvent standard pesticide mix to determine an optimal DART heater temperature of 175 ° C for the pesticide mixture and 250 ° C for targeted carbendazim analysis. Several of the pesticides could be detected as low as 10 ppb directly from the juices using this method and carbendazim was directly detected out of orange juice as low as 2 ppb with limit of quantitation of 15 ppb.
An identical DART set-up was coupled with a Q Exactive benchtop Orbitrap mass spectrometer. The Q Exactive provided a versatile MS platform to both rapidly screen with low limits of detection and preselect ions of interest generating high resolution targeted MS/MS data for comparison with the QTRAP MRM data. Limits of detection using the DART Q Exactive quantitative method were below 10 ppb for the 10 pesticides in the mixture, as well as for carbendazim directly analyzed from fruit juices. Of all of the orange juices screened for carbendazim three juices all purchased in the EU tested positive at levels ranging from < 5 ppb up to 21 ppb.

PTu-147
11:10 – 12:20

Study of fragmentaion behaviors for atmospheric negative ion adducts of phenylalanine homologues using atmospheric pressure corona discharge ionization mass spectrometry

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Keywords:
phenylalanine, radical, fragmentation, negative ion

Novel aspects:
Information about fragmentation behaviors for deprotonated molecule of phenylalalnie homologues was obtained, and areaction of hydrogen abstract induced by radical speces was found.

Abstract:
Interpretation of fragmentation behaviors for both (de) protonated analytes $[M\pm H]^{\pm}$ using collision induced dissociaton (CID) spectra makes it possible for the structual elucidation of analytes M, which is useful to identify unknown analytes in mass spectrometry. However, it is difcult to obtain systematic information of fragmentation behaviors for $[M-H]^-$ compared with those for $[M+H]^+$. We have recently established an atmospheric pressure corona discharge ionization mass spectrometry (APCDI-MS) that results in soft ionizaton in negative-ion mode using various atmospheric negative ions R (e.g., O_2^-, CO_x^- and NO_x^-) to form atmospheric ion adducts $[M+R]^-$. The adduct ions $[M+R]^-$, furthermore, can be fragmented to deprotonated analytes $[M-H]^-$ and other product ions. Here we performed the CID experiments for atmospheric ion adducts of phenylalanine homologues all of which are aromatic compounds having an amino and carboxyl groups, using APCDI-MS. The CID data obtained provided the information about the fragmentation behaviors for $[M+R]^-$ and $[M-H]^-$ for individual phenylalanine homologues.

Mass spectra were acquired on a TSQ7000 triple-quadrupole mass spectrometer (Thermo Fisher Scientic, San Jose, CA) . The discharge ion source used contained a needle (ca. 1 μm in the tip curvature radius) and a metalic orice plate, which were separated by a gap of 3 mm. DC needle voltages and needle angle with respect to the orice axis were varied over the range of -1.9--3.9 kV and $0-\pi/2$ rad, respectively. A micro-ceramic heater was placed between the needle and the orice plate in order to vaporize condensed-phase analytes. Discharge ionization was performed under atmospheric pressure in laboratory air with relative humidity of 50-70 % at 298 K. Collision gas and energy (lab.) for CID experiments used were argon at 2.0×10^{-3} torr and 5 -25 eV, respectively.

Gaseous L-phenylalanine (L-Phe) was ionized by various atmospheric negative ions R^- (=O_2^-, HCO_3^-, $HCOO^-$, CH_3COO^- and COO^-(COOH)) , and resulting in the formation of atmospheric ion adducts $[L\text{-}Phe+R]^-$. The CID of all the adducts $[L\text{-}Phe+R]^-$ except for $[L\text{-}Phe+CH_3COO]^-$ brought about the production of deprotonated molecule $[L\text{-}Phe\text{-}H]^-$ and/or the atmosperic ions R^-, formed via the dissociation accompanied by proton abstraction from L-Phe by R^- and a simple dissociation of the adduct $[L\text{-}Phe+R]^-$, respectively. Which product ion peak can have a higher intensity was dependent on the proton afnities between $[L\text{-}Phe\text{-}H]^-$ and R^-. When the proton afnity of R^- is higher than that of $[L\text{-}Phe\text{-}H]^-$, R- can efciently abstract a proton from L-Phe to form dominant product ion $[L\text{-}Phe\text{-}H]^-$. The adduct $[L\text{-}Phe+CH_3COO]^-$, in contrast, resulted in the formation of abundant $[L\text{-}Phe\text{-}3H]^-$ and less abundant $[L\text{-}Phe\text{-}H]^-$ and CH_3COO^-. This result suggests that CH_3COO^- is a biradical anion species CH_3COO^- and can abstrat not only a proton but also two hydrogen atoms from L-Phe.

The gaseous ionic species R^- described above bind to L-Phe homologues M, D-phenylalanine (D-Phe) , L-homophenylalanine (HPA) and L-phenylglycine (PG) , to form $[M+R]^-$. The fragmentation behaviors of $[M+R]^-$ for the fragmetation of $[M-H]^-$ and/or R^-, or $[M-3H]^-$ were the same as that of $[L\text{-}Phe+R]^-$.

CID of $[L\text{-}Phe+R]^-$ and $[M+R]^-$ with higher collision energies provided the ionformation about the fragmentation behaviors of deprotonated analytes ($[L\text{-}Phe\text{-}H]^-$ and $[M-H]^-$) for individual analytes. $[L\text{-}/D\text{-}Phe\text{-}H]^-$ could be fragmented via two different processes, i.e., successive losses of NH_3 and CO_2 to form $C_6H_5(CH)_2COO^-$ and $C_6H_5(CH_2)^-$ ions, respectively, and the loss of toluene for the formation of NH (CH) COO^- ion, in which the fragmentation behaviors were independent of L and D bodies. The precursor ion $[HPA\text{-}H]^-$ led to the formation of product ion $NH_2CH_2COO^-$ via the loss of styrene, whereas CID of $[PG\text{-}H]^-$ brought about the loss of CO_2 and resulting product ion $C_6H_5C^-(H)(NH_2)$.

PTu-148
13:30 – 14:40

Unusual radial cation formation of arginine by atmospheric pressure corona discharge ionization (APCDI)

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Keywords:
Radical cation, arginine, APCDI, hydrogen loss

Novel aspects:
Unusual formation of radical cation of most basic amino acid arginine was observe in APCDI method, and its mechanism (s) was discussed.

Abstract:

Introduction

It has been well known that ambient ionizations such as DESI, ASAP and any other API methods give protonated molecules $[M+H]^+$ in positive ion mode, although the resulting analyte ions are according to the thermochemical properties of analyte. The protonated molecules generate via ion/molecule reactions such as proton transfer from reactant ions RH^+ to analyte molecules M, under atmospheric pressure conditions. The proton transfer reactions depend on the proton affinity (PA) or gas-phase basicity (GB) of analytes and reagent gas molecules R. Here we performed the experiments of atmospheric pressure corona discharge ionization (APCDI) of all the twenty different amino acids. Amino acids commonly have basic and acidic functional groups called as N- and C-terminal side, respectively. Furthermore, the amino acids have several physicochemical properties such as hydrophobic, hydrophilic, aromatic, sulfur-containing, acidic, basic and neutral, according to the nature of side chains. Although it would be expected that most basic amino acid arginine (Arg) gives a dominant peak corresponding to protonated molecules $[M+H]^+$ in positive-ion APCDI experiments, the APCDI mass spectrum of gas-phase Arg molecules gave the dominant peak corresponding to radical cation $M^{+\cdot}$ at *m/z* 174 in the APCDI mass spectrum. In the present paper, we discuss about the mechanism (s) of the formation of radical cations of basic analytes, as well as Arg molecules.

Experimental

Mass spectra were acquired on a TSQ-7000 triple-quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Atmospheric pressure corona discharge ionization (APCDI) source used contained a needle (ca. 1 um in the tip curvature radius) and the orifice plate of the mass spectrometer, which were separated by a gap of 3 mm. A micro ceramic heater was placed between the point-to-plane electrodes to rapidly vaporize condensed-phase analytes. DC voltage applied to the needle was +3.4 kV. The orifice temperature was varied in the range of 333-373 K. The discharge experiments were performed in the laboratory ambient air under atmospheric pressure with relative humidity of 45-65 % at 298K. The analytes used were Arg, aniline, 5-amino salicylic acid (5-ASA), and related compounds.

Results

The dominant analyte ion formed in APCDI mass spectra of organic compounds was generally protonated molecule $[M+H]^+$. The formation of $[M+H]^+$ is most likely to be attributed to reaction (1) involved in the oxonium ion H_3O^+ which is a major atmospheric ion species produced in the discharge area. That is, the formation of atmospheric ions adduct $[M+H_3O]^+$ is initially occurred, and subsequently the protonated analyte $[M+H]^+$ is generated by the loss of water molecule H_2O due to proton abstraction from H_3O^+ by M.

$$M + H_3O^+ \rightarrow [M+H_3O]^+ \rightarrow [M+H]^+ + H_2O \quad (1)$$

Because of ionization processes including proton transfer from H_3O^+ to M, only the analytes with proton affinities higher than H_2O (691 kJ mol^{-1}) can be ionized.

It was found, however, that several analytes, Arg, aniline and 5-ASA, are ionized as not only $[M+H]^+$ but also radical cations $M^{+\cdot}$. It has been well-known that $M^{+\cdot}$ can be formed by electron emission due to electronic excitations of M (reaction 2), i.e., electron ionization (EI).

$$M + e^- \rightarrow M^{+\cdot} + 2e^- \quad (2)$$

The CID of the $M^{+\cdot}$ for aniline resulted in the formation of the product ion $C_5H_6^{+\cdot}$ via the loss of HCN. This fragmentation behavior was in agreement with $M^{+\cdot}$ obtained with EI, indicative of that $M^{+\cdot}$ formed with APCDI has the same structure as that with EI. On the other hand, the CID pattern of $M^{+\cdot}$ for arginine formed with APCDI was *inconsistent* with that by EI.

PTu-149
11:10 – 12:20

Theoretical and experimental studies of ionization of explosive compounds using dielectric barrier discharge ionization

Hirotaka Yabushita[1], Hirofumi Nagao[1], Toshinobu Hondo[1], Michisato Toyoda[2]

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Keywords:
explosive, dielectric barrier discharge ionization, fragmentation, matrix effect, quantum chemical calculation

Novel aspects:
We investigated effect of additives on dielectric barrier discharge ionization of explosive compounds not only experimentally but also theoretically by using quantum chemical calculation.

Abstract:
Method development for rapid on-site detection and identification of criminal compounds is important to find scientific evidence of terrorism and crimes. Minimized sample preparation and high sensitive method are required.

Recently, several researchers have studied plasma discharge atmospheric pressure ionization (API) mass spectrometry [1-3] for high sensitive analysis of explosive compounds. In order to apply their method to practical crime scene investigation, effect of common chemical background should also be addressed.

X. Zhao and J. Yinon reported that the electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectrometry of nitro ester compounds were affected by impurities in the analytical system to form a variety of adduct ion instead of molecular ion, which made it difficult to interpret the spectrum. To solve this problem, they introduced ammonium chloride to post-column. The addition of ammonium chloride led to formation of stable chlorine adduct ion and improved detection limit of nitrate ester [4] .

The addition of the compound reacting with target to form stable ion also should be useful for plasma discharge API method. In order to investigate ionization reactions of explosive compounds, we designed dielectric barrier discharge (DBD) ionization source in house which was attached to FT-ICR mass spectrometer (Apex III 4.7T, Bruker Daltonics Inc. Billerica, MA, U. S.) . Sample was placed on the glass plate in ambient atmosphere and vaporized by halogen lamp as heater. Vaporized gas was aspirated into DBD source through 0.8mmI.D. by 50mmL of stainless-steel capillary that maintained 100 degrees Celsius. Helium gas was controlled by mass flow controller (D213, HORIBA STEC, Kyoto, Japan) . Dichloromethane (DCM) was introduced through the stainless-steel capillary.

Both meta-dinitrobenzene (m-DNB) and para-dinitrobenzene (p-DNB) gave similar complex mass spectra consisting of several reacted ions under the condition without DCM. By DCM addition, simple chlorine adduct ion without significant fragmentation was observed for m-DNB. The identification efficiency and sensitivity were improved. In contrast, the ionization of p-DNB was drastically suppressed by DCM addition.

This result shows that addition of DCM may improve m-DNB detection performance but cause false negative result for p-DNB detection. We assume the orientation of nitro-group changes chlorine affinity that improves m-DNB detection performance but not for p-DNB. We will report further study of chlorine affinity difference by using quantum chemical calculation.

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PTu-150
13:30 – 14:40

Quantification of six anti-diabetic drugs in human plasma by indirect spray FT ICR mass spectrometry

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Keywords:
Ambient ionization ; Indirect spray ; Anti-diabetic drugs ; Quantification ;

Novel aspects:
A a novel ambient ionization technique named indirect spray are used for quantification.

Abstract:
Indirect spray is a novel ESI-based ambient ionization technique. It has similar geometry arrangement as TM-DESI, instead of using a mesh, membrane funnel plate is used as sample loading substrate. No pneumatic assistance is needed in indirect spray. When charged solvent droplets were sprayed onto the membrane funnel and the deposited analytes dissolves in, then a secondary electrospray i.e. indirect spray is induced. Indirect spray is applicable for direct detection of different types of compounds, from small drug molecules to protein, using a small volume of sample (typically 50 nl) . It has been proved indirect spray is of higher sensitivity than TM-DESI. In this work, we validate the ability of indirect spray for quantitative analysis. Six chemically diverse anti-diabetic drugs, including acarbose, pioglitazone hydrochloride, acetohexamide, repaglinide, insulin and vildagliptin, were examined. Different drugs have different limit of detection (LOD) ranging from 0.5 fmol to 2 fmol. Dynamic range of this technique was typically three or four orders of magnitude. Precision and accuracy values for quality control vildagliptin samples range from 1.7 % to 14 % and from 6.5 % to -15 % respectively. Extending the current indirect spray analysis to biological background is in processing. SPE sorbent attached membrane funnel will be fabricated to eliminate the matrix effect. Different methods of adding internal standard (vildagliptin-d 3) will be tested. Inter-and intra-day precision and accuracy and LOD will also be investigated for vildagliptin in plasma.

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PTu-151
11:10 – 12:20

Physical process hierarchy in ion movement inside a gas dynamic interface for the mass-spectrometer with ambient ionization.

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Keywords:
ambient ionization, gas dynamic interface, ion mobility,

Novel aspects:
the model to account simultaneously the effect of gas dynamic, DC and RF electric fields in a gas dynamic interface including the effect of ion inertia

Abstract:
Ion transport inside the gas dynamic interface of the mass-spectrometer with ambient ionization is one the most important factors affecting losses in ions and hence, the detection limit and instrument discriminations. One needs to simultaneous account for gas dynamic forces, DC and RF electric fields to provide correct description of the interface processes. Electrode positions can change the structure of gas dynamic flows, so generally, one should consider a self-consistent task.

For an empty chamber or for electrode positions affecting poorly on gas flows, the most important parameter is the ratio of the chamber length L to the Mach barrel length $L_M = 2/3\ d\ (P/P_0)^{1/2}$, where d is the inlet diameter, P and P_0 are the static pressures at the interface and at the inlet respectfully. Pulsations of the shock wave position and macro turbulence are inherent for a 'short' chamber with $L < 2\,L_M$; on the contrary, a stable gas jet with the strict position of the shock wave and the automodel shape down the Mach barrel is typical for $L >> L_M$. Usually, the Mach barrel length $L_M = 2\ 6$ mm.

The second dimension parameter is important for a 'long' chamber, the one characterizing the jet divergence. If the automodel structure is well organized, the jet becomes several times wider along the chamber, gas if looses its velocity, and the region of micro turbulence appears ; it can be observed, e.g., at L~40 mm for P=1000 Pa and d = 0.5 mm. However, the jet is nearly parallel and the gas velocities are about 400 500 m/s reaching all the way to the skimmer at an interface pressure of 200 Pa.

Accounting the role of electric field, one can try to consider the ion velocity as a vector sum of the gas velocity $\mathbf{V_g}$ and electric velocity in a form $\mathbf{V} = \mathbf{V_g} + \mathbf{V_E} = \mathbf{V_g} + K^*\mathbf{E}$, where **K** is the mobility, and **E** is the field. This approach works well for the atmospheric region, but it neglects the time necessary for an ion to accelerate or decelerate ; one needs to account correctly this time depending on the ion mass, when working in the intermediate vacuum pressures. This mobility approach limits the spatial and time gradients of the electric and gas velocity fields, that may be considered. Extention of the validity range may be better accounted by a Stocks criterion $Sk = t_{in}/t_0$, where t_{in} is the ion inertia time, $t_{in} = K^*M/Z$, M/Z is the mass to charge ratio, and $t_0 = \mathbf{E}^*\ (d\mathbf{E}/d\mathbf{r})^{-1} * \mathbf{V}^{-1} + \mathbf{E}^*\ (d\mathbf{E}/dt)^{-1}$ characterizes the changes in field along the ion trajectory.

To account for RF field role requires a more complex approach. We have shown that the effect of pseudo-potential, i.e. ion repulsion from the spatial gradient of RF filed, appears only due to the phase difference between the momentary value of the ion velocity and the field ; this difference is maximal at high vacuum and decreased with the increase of the gas density. This allows one to predict and calculate ion repulsion from spatially non-uniform RF fields for the given pressure, frequency, RF magnitude, and electrode structure.

PTu-152
13:30 – 14:40

Using Thin Layer Chromatography Spray (TLC spray) Ionization Mass Spectrometry to Analyze Drugs in Whole Blood

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Keywords:
Thin layer chromatography, mass spectrometry, spray ioinization

Novel aspects:
A new TLC/MS technique, TLC spray, was developed for rapidly characterizing the chemical compounds on the TLC plate.

Abstract:
Thin layer chromatography (TLC) is an analytical tool commonly used for sample purification and separation. To characterize the separated chemical compounds on TLC plate, coupling TLC with mass spectrometry (TLC/MS) seems to be the most efficient approach. Since the analytes are buried in the stationary phase bed, the development of an efficient sampling method is crucial in detecting the analytes by TLC/MS. In this study, a new TLC/MS technique TLC spray was developed for rapidly characterizing the chemical compounds using the TLC plate. The drugs spiked in the whole blood were successfully detected by this TLC spray mass spectrometry approach.

The design of the TLC spray is similar to that of a direct electrospray probe except the fine rods of DEP. The tip end of a TLC plate (silica gel on aluminum sheet) was cut to a sharp triangle. The small amount of whole blood sample was spotted on the TLC plate. A syringe pump was used to apply the mobile phase (acidic methanol solution) through a capillary onto the TLC plate to separate the analytes in the sample spot. After applying high voltage on the TLC sheet, electrospraying was generated at the tip of the TLC plate. The analytes eluted out of the TLC plate were ionized by electrospray ionization and detected by a quadrupole/time-of-flight mass spectrometer. The TLC spray MS was used to characterize ketamine, methamphetamine, and beta-2-adrenergic receptor agonist spiked in whole blood. Since blood cells and large molecules were too heavy to be carried in the mobile phase, they stayed at the original sample spot during development. Only small compounds such as spiked drugs were carried to the end of the TLC plate. In this preliminary study, the spiked drugs were detected in whole blood and the detection limit of methamphetamine by TLC spray MS is estimated to be approximately 1 ppm.

PTu-153
11:10 – 12:20

Ambient surface mass spectrometry of polymers and molecules using PADI - Optimising parameters, improving repeatability and damage effects

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Keywords:
polymers, plasma, depth profiling, PADI

Novel aspects:
Repeatable ambient surface mass spectrometry of polymers, and initial investigation of depth profiling capability.

Abstract:
Reliable and repeatable measurements are essential for the strong uptake of any analytical technique. Ambient surface mass spectrometries have demonstrated exciting and revolutionary measurement capability but for wider acceptance in industry they need a secure metrology foundation. Plasma sources are increasingly being used for ambient mass spectrometry since the development of DART (direct analysis in real time) in 2005 and subsequent imaging methods such as PADI (plasma assisted desorption ionisation) and LTP (low temperature plasma) . Here, we commence establishing the essential metrology for PADI, which has already been shown to be successful at analysing pharmaceuticals [Anal. Chem. 2007 ; 79, 6094] and personal care products [Analyst, 2011 ; 136, 3274] .

The PADI system is an atmospheric pressure RF generated helium plasma coupled to a Thermo LTQ-Orbitrap Velos mass spectrometer. We conduct a systematic study of the effects of geometry, RF power and gas flow rate on the relative signal intensities, sensitivity and repeatability using molecular (valine) as well as polymer (PET, PMMA, PTFE and PLA) surfaces.

We conduct a detailed study of the instrumental contributions to the spectral repeatability, including the intrinsic noise in the mass spectrometer. Firstly, we identify and separate out the contributions to the repeatability (relative standard deviation) of the PADI peak intensities. We show that the Orbitrap has a noise distribution approximately as expected for random noise so that at intensities of above 10^8 'counts' it is less than 2.2%. The standard deviation of the PADI signal intensity is proportional to the signal intensity which is clearly not random but related to systematic effects in the source operation. Optimisation of the plasma source is essential for ensuring robustness and reliability. We show how the signal varies with geometry, helium flow rate and plasma power. Thermal imaging of the sample surface shows that the temperature rises approximately linearly with plasma power and at 19 W is 70 °C. To reduce the effects ofdamage for surface sampling, we recommend keeping the surface temperature below 40 °C by operating at less than 15 W. General guidance is given for practical analysis.

Importantly, PADI can analyse a wide variety polymers. For this, it is essential that the plasma probe is not near a grounded electrode. Analysis of polymers gives mass spectra with repeating series containing different fragments and adducts. These are identified using the high-resolution and mass accuracy of the Orbitrap MS. Preliminary studies of sample surfaces after PADI analysis show that the plasma can change the chemical make up of the surface leading to damage. With the recommended operating parameters, the plasma erodes the polymer sample at a rate of 0.87 nm s^{-1} (for PMMA) . Quantitative analysis of the eroded crater bottom by x-ray photoelectron spectroscopy (XPS) shows the chemical damage is less than 10%. This shows the potential for polymer depth profiling. Similarly, depth profiles for molecules are obtained. The potential for PADI for mass spectrometry imaging for a wide range of applications will be discussed.

PTu-154

13:30 – 14:40

Thermal Dissociation Atmospheric Chemical Ionization Mass Spectrometry with a Miniature Source for Selective Trace Detection of Dimethoate in Fruit Juices

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Keywords:

TDCI；Ionic liquid；dimethoate；without any sample pretreatment

Novel aspects:

Instead of toxic solvents and high-voltage or high electric field used in ambient ionizations, ionic liquid, a "green solvent ", was employed to directly generate reagent ions in the TDCI process

Abstract:

A miniature thermal dissociation atmospheric chemical ionization source (TDCI) coupled with a LTQ-MS has been developed for rapid trace detection of dimethoate in fruit juices. Instead of toxic solvents and high-voltage or high electric field used in ambient ionizations, ionic liquid, a "green solvent ", was employed to directly generate reagent ions in the TDCI process. The ionization was occurred by the followed proton or charge transfer reactions with the analytes presented in the matrices of the raw samples under ambient conditions. Trace amounts of analyte in the fresh orange juices has been quantitatively detected, without any sample pretreatment or aid of high-pressure gas. A low limit of detection (LOD=8.76×10^{-11} g/mL) and acceptable relative standard deviation (RSD=3.5%-8.5%) , as well as reasonable recoveries (90-112%) , were achieved with this method for direct detection of dimethoate in complex orange juice samples. The average analysis time for each single sample was less than 30 seconds. These experimental results showed that TDCI-LTQ-MS is a powerful tool for the fast trace detection of pesticide residue such as dimethoate in complex viscous fruit juices, with the advantage of high sensitivity, high speed, ease of operation, and so on. Because of no organic solvents pollution and invasiveness to the analytes and environments, the technique has promising applications for inline quality monitoring in the food safety.

PTu-155
11:10 – 12:20

Mass analysis of the ions generated by corona discharge ionizers in ambient air

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Keywords:
corona discharge, atmospheric pressure ionization mass spectrometer, air ions, cluster ions

Novel aspects:
The evolutions of the postive and negative ions generated by corona discharge ionizer in ambient air were measured using API-MS.

Abstract:
Corona discharge ionizers are widely used to generate ions in air. They are typically installed in commercial electrical appliances such as air cleaners and air conditioners because negative ions have been reported to reduce the levels of particulates, airborne microbes, odors and volatile organic compounds in indoor air. However, the detailed mechanisms by which negative air ions contribute to improving indoor air quality are not clearly understood.
Information on the ion composition produced by such devices is essential if we are to understand the role of ions in air cleaning processes. In order to investigate the characteristic compositions of the ions generated by corona discharge ionizers that are used in commercial air cleaner, mass spectrometric measurements were conducted using an atmospheric pressure ionization mass spectrometer. The ionizer was placed in front of the sampling orifice of the mass spectrometer. The distance between the ionizer and the orifice was varied to see the effect of ion reaction time in ambient air.
In positive ion mass spectra, major ions were $H_3O^+(H_2O)_n$ and $NH_4^+(H_2O)_n$. With increasing reaction time, $NH_4^+(H_2O)_n$ became dominant as a result of proton transfer reaction from $H_3O^+(H_2O)_n$ to NH_3. Unidentified ion peaks often appeared in positive ion mass spectra. Some kinds of VOCs that have large proton affinities are probably responsible for those ions. Negative ions in the measured mass spectra included $O_2^-(H_2O)_n$, $NO_2^-(H_2O)_n$, $CO_3^-(H_2O)_n$, and $NO_3^-(H_2O)_n$. Among these ions, $NO_3^-(H_2O)_n$ were observed to persist for long time while other ions declined with increasing reaction time. $NO_3^-HNO_3(H_2O)_n$ were also observed to form as stable negative ions in the air. The presence of this cluster ion indicates that a large amount of nitric acid is formed by negative corona discharge in ambient air. The reaction of NO_2 with OH leads to the formation of nitric acid. Both NO_2 and OH are byproducts generated by negative corona discharge in air and they play important role in the formation of stable negative ions. In addition, ion peaks probably due to HCO_3^- ions and also to the ions of some organic compound were observed in negative ion spectra. The relative abundance of ion species varied depending on the condition of the air in the laboratory.

PTu-156
13:30 – 14:40

Rapid Screening of Camphor Wood by Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry

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Keywords:
Surface desorption atmospheric pressure chemical ionization, mass spectrometry, principal component analysis, camphor, cluster analysis

Novel aspects:
Fast, low-cost, reliably method to distinguish camphor wood from fake camphor wood

Abstract:
It is growing interest to distinguish non-camphor wood and authentic camphor wood quickly, cheaply and reliably. Appearance and smell are used typically, but are unreliable. Although polymerase chain reaction (PCR) is the gold standard, it is a slow method. In this work, a surface desorption atmospheric pressure chemical ionization (SDAPCI) source is used to obtain the mass spectra of 5 camphor wood types and one unspecified wood type without pretreatment. The wood types are analyzed by both principal component analysis (PCA) and cluster analysis (CA). The characteristic ions for camphor and linalool are identified from the PCA loading plot. The single-scan method distinguishes the three strongest peaks: 1) the camphor molecular ion, 2) the camphor-water adduct and 3) the linalool molecular ion from the wood surface, and the tandem mass scan confirms the identities. This method classifies 30 samples by category and quality without pretreatment, is sensitive and is fast (less than 3 min per sample).

PTu-157
11:10 – 12:20

Hydrocarbons Analysis by Desorption Atmospheric Pressure Chemical Ionization

Fred Paul mark Jjunju Jjunju[1,2], Badu_Tawiah K Abraham[2], Li Anyin[2], Roqan S Iman[1], Graham R Cooks[2]

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Keywords:
Mass spectrometry, Ambient ionization, DAPCI, Petroleum hydrocarbons

Novel aspects:
DAPCI ionization source coupled with miniaturized mass spectrometers could provide the first hand-held, in-field instrumentation for non-volatile compound characterization for the petroleum industry.

Abstract:
The advent of ambient ionization techniques has enabled the mass spectrometric analysis of chemical compounds from surfaces in their native environment. Such methods are particularly useful for *in situ* analysis since the sample is analyzed without prior preparation. Among the ambient ionization techniques are the plasma-based methods of which desorption atmospheric chemical ionization (DAPCI) is an example. In DAPCI, a corona discharge is directed at a surface using a carrier gas at a low flow rate to desorb and ionize analyte (s) . Interestingly, a charge exchange reagent (e.g. naphthalene) can be doped into the gas stream to effect electron transfer reactions. This mode of DAPCI is useful for samples, such as hydrocarbons, which have low proton affinities.

Two types of DAPCI experiments are presented based on the mechanism of sample ionization i.e. proton or charge transfer. The background mass spectrum for DAPCI experimental conditions utilizing only a corona discharge and nitrogen carrier gas showed predominantly the presence of high abundant water cluster H $[H_2O]_2^+$andH $[H_2O]_3^+$ ions at *m/z* 37 and 55 respectively. Analysis of petroleum constituents such as hydronaphthalene, thiophenes, alkyl subsitituted benzenes, pyridines, fluorenes, and polycyclic aromatic hydrocarbons (PAH) model compounds using the water clusters as DAPCI probe ions produced mass spectra which were dominated by protonated molecules $[M+H]^+$. On the other hand, molecular radical cations were observed when the nitrogen carrier gas was doped with naphthalene as a charge transfer reagent. In this case, the DAPCI probe ions were naphthalene radical cations, which upon interaction with the analyte present at the surface ionized it through charge exchange. This mode of sample ionization extended the applicability of DAPCI to other petroleum constituents (e.g. higher molecular PAHs such as chrysene) which could not be analyzed in the proton transfer mode of operation. The thermochemistry governing the individual ionization processes are discussed. Aside from the two main chemical reactions (proton vs. electron transfer) observed under the two different DAPCI modes, certain specific compound classes (e.g. hydronaphthalenevs. thiophenes vs. alkyl substituted benzenes) of petroleum constituents underwent different reactions. These differences were especially marked under the DAPCI condition in which no charge exchange reagent was utilized. For example, in addition to analyte protonation, hydride ion elimination was also observed for the hydronaphthalenes compared with oxidation reactions for tetramethyl- and pentamethyl-substituted benzenes. Intact molecular (or pseudomolecular) ions were mostly observed irrespective of the ion form (i.e. $[M+H]^+$, $M^{\cdot+}$ or $[M-H]^+$) and the chemistry involved, with the exception of low molecular weight *n-alkanes* which produced extensive fragmentation after hydride abstraction.

PTu-158
13:30 – 14:40

Rapid Detection of Bacterial Lipids, Metabolites and Signalling Compounds by Liquid Extraction Surface Analysis Mass Spectrometry

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Keywords:
Microorganisms, Acylated homoserine lactones (AHLs) , Biofouling, Metabolites, Liquid extraction surface analysis (LESA) .

Novel aspects:
New methods employing liquid extraction surface analysis-mass spectrometry, have detected bacterial metabolites associated with biofilm production directly from the cell surface.

Abstract:
Introduction: Microorganism growth resulting in biofouling of coated steel building materials is a growing concern for the multi-billion dollar Australian industry. Proliferation of microorganisms on these materials is facilitated by the creation of a protective extracellular matrix or 'biofilm' and is associated with a range of problems including sick building syndrome, corrosion of materials, and discoloration. Despite its importance, very little is known about the process of biofilm development on such surfaces or the organisms involved. Environmental adaptation and biofilm formation has also been associated with microbial cell-cell communication, mediated by *N*-acyl-homoserine-lactones (AHLs) and interference with AHL signalling has been proposed as a mechanism to control microbial fouling. Targeted strategies to combat biofouling in this manner therefore require the development of rapid methods for detecting microorganisms present on these surfaces and identifying metabolites as well as signalling compounds associated with biofilm formation.

Method: Filtered methanol extracts of *Pseudomonas aeruginosa* cultures pipetted (2 µL) onto an Omni Slide™ hydrophobic array (Prosolia Inc., Indiannapolis, IN, USA) and *Chromobacteriumviolaceum* cultured on agar were analysed by liquid extraction surface analysis (LESA) using an automated, chip-based nanospray source (TriVersa NanoMate®,Advion, Ithaca NY,USA) coupled to a triple quadrupole linear ion trap mass spectrometer (QTRAP®5500, AB Sciex, Foster City CA, USA) . Analyst® 1.5.1 software (AB Sciex, California, USA) was used for spectral acquisition and typical experimental conditions were ; spray voltage (1.4 kV) , N_2 delivery gas (0.3 psi) and MeOH : $CHCl_3$ (2 : 1) with 0.1 % formic acid (v/v) as the solvent system. A total volume of 2 µL of solvent was used for each experiment with 1 µL being dispensed 0.6 mm above the surface by an automated pipette tip. The solvent forms a liquid-surface junction facilitating liquid extraction of analytes into the solvent that is then subjected to electrospray ionisation through a nanospray chip.

Preliminary Data: Liquid extraction surface analysis-mass spectrometry (LESA-MS) by means of a TriVersa NanoMate® ionisation source coupled to a QTRAP® 5500 mass spectrometer has proven to be a fast, simple and effective method in the detection of bacterial metabolites from solvent extracts and directly from a cell culture surface. Unlike other ionisation techniques including electrospray ionisation or matrix-assisted laser desorption ionisation, LESA-MS is able to directly analyse the bacterial cells *in situ* making the technique extremely fast and simple. Utilising this technique we are able to perform lipid profiling that may allow us to distinguish between different species of microorganisms. With this knowledge we can then direct our analyses to known metabolites for those species. Employing precursor scans targeting the common AHL product ion at *m/z* 102, positive ion LESA-MS analyses of *P. aeruginosa* solvent extracts were able to detect a range of hydrolysed AHLs including 3-oxo-C_{10}-HS and 3-oxo-C_{12}-HS. These AHLs are bacterial quorum-sensing molecules associated with biofilm formation.[1] Negative ion LESA-MS analyses of *C.violaceum* cells cultured on agar were able to chemically identify the characteristic purple pigment violacein, an antibacterial compound produced in response to the presence of AHLs.[2] Violacein was detected as an $[M-H]^-$ ion at *m/z* 342.1 with MS/MS spectra exhibiting characteristic product ions at *m/z* 298.1, 209.1, and 157.0. [2]

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PTu-159
11:10 – 12:20

A hydrodynamically optimized nanoelectrospray source with 100% transmission

Matthias Pauly[1], Mario Sroka[2], Stephan Rauschenbach[1], Julius Reiss[2], Gordon Rinke[1], Alyazan Albargash[1], Jörn Sesterhenn[2], Klaus Kern[1]

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Keywords:
Atmospheric Pressure Ionization (API) , Electrospray ionization (ESI) , ion transfer in vacuum, MS instrumentation

Novel aspects:
Our new interface shows transmission into vacuum of up to 100 % of the ions generated at a nanoelectrospray emitter.A much larger acceptance volume as compared to conventional interfaces is observed.

Abstract:
Ions generated in atmospheric pressure ionization (API) sources, including Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) , have to be transferred into vacuum in order to be analyzed by mass spectrometry (MS) , ion mobility spectrometry (IMS) or to be deposited on a substrate (soft landing) . The coupling of an API source to a vacuum chamber is made via an interface, typically a transfer capillary or a pinhole of small diameter, as pumping speed is limited. Present interfaces are very inefficient, as they only transfer less than 5 % of the generated ions into vacuum. Nano-ESI sources can reach transmissions up to 10%.

To improve the performance of the vacuum transfer, we optimized the geometry of the transfer capillary, taking all influences on the ion motion into account : electrostatic forces from applied voltages and space charge forces as well as hydrodynamic drag forces.

Our new interface shows transmission into vacuum of up to 100% of the ions generated at a nanoelectrospray emitter for analyte concentration below 10^{-3} M. For high concentrations and high electrospray voltages a threshold current in the range of 2 -40 nA is reached beyond which the transmission decreases. Further, a much larger acceptance volume as compared to conventional interfaces is observed. Based on computational fluid dynamics calculations of the gas flow within the capillary and ion motion simulations, we show that hydrodynamic forces of an optimized gas flow collimate the ion cloud, which effectively counteracts the space charge forces that are the main reason for ion losses.

This ion source represents a major improvement over present nanospray sources in terms of transmission. In particular measurements requiring high sensitivity or high ion flux will profit from this development. Further the approach of hydrodynamic optimization will allow improving other atmospheric pressure ion sources significantly.

PTu-160 Direct analysis of asphaltene by DART FT MS

13:30 – 14:40

Hugues PREUD'HOMME[1], Felipe CARDOSO[2,3], Bruno GRASSL[4], Brice BOUYSSIERE[1], Herve CARRIER[2]

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Keywords:
asphaltenes, DART, FT MS

Novel aspects:
Direct analysis of asphaltene obtained at high pressure and high temperature by DART FT MS

Abstract:
Direct Analysis in Real Time (DART) is an ambient ionization technique undergoing rapid development. With minimal sample treatment, ionization of analyte molecules outside the mass spectrometry instrument in the ordinary atmosphere is feasible. The ionization approach relies upon the fundamental principles of atmospheric pressure chemical ionization and here we are able to generate ions even for saturated hydrocarbon. We discussed here firstly of optimization of DART source and FTMS setting parameters in order to achieve routinely fingerprint of Petroleum fluids, aggregates or deposition. They are complex mixtures containing thousand of different compounds among which asphaltenes. Aggregation and deposition of asphaltenes during crude oil production, transportation and treatment may cause serious problems as reported in numerous papers. This very complex matrix is not fully identified from a chemical point of view. Indeed asphaltenes are still defined by a solubility class (IP 143 for example) , being soluble in aromatic solvents (toluene, xylene,) and insoluble in paraffinic ones (heptanes, pentane, ...) . However, the solid material extracted from crude oil according to the above mentioned protocol contains the higher concentration in metals such as nickel and vanadium. In order to better understand asphaltenes behavior and their chemistry, a DART ion source and FT MS acquisition has been performed.
The experimental set up used to induce asphaltenes flocculation is able to work up to 100 MPa and 473° K. It allows investigations with recombined oil or live oil fluids. In the work presented here, asphaltenes deposits engendered by pressure drops similar to those occurring during oil production have been obtained and collected onto stainless steel filters. The solid materiel has been directly analyzed by DART FT MS .

PTu-161

11:10 – 12:20

BRIDGING BETWEEN LIFE SCIENCES AND MATERIAL SCIENCES: MASS SPECTROMETRIC PROTEIN AND METABOLITE ANALYSIS FOR THE PRODUCTION OF BIO-BASED CHEMICALS

Michiel Akeroyd, Rob van der Hoeven, Rene Verwaal, Stefan Turk, Fredoen Valianpour, Marcel Tilborg, Maurien Olsthoorn

DSM Biotechnology Center / Delft / The Netherlands

Keywords:
Industrial Biotechnology, Synthetic Biology, Proteomics

Novel aspects:
Mass spectrometry aided development of industrial bio-based chemical production, Fermentative production of bio-chemical compounds using synthetic biology, Mass spectrometric intra-cellular pathway monitoring at metabolite and protein level, Bioinformatic route scouting

Abstract:
DSM is driving to create sustainable high value products through connecting its competences in Life Sciences and Material Sciences. Recent examples of this strategy are the production of succinic acid in *S. cerevisiae* and 6-amino caproic acid (6-ACA) in both *C. glutamicum* and *E. coli* using biotechnological processes. The first process enables large scale renewable production of a natural occurring metabolite as a building block for high value polymers. The second process is the bio-production of a non-natural compound, the building block for Nylon-6.

Mass Spectrometry was one of the key techniques used for analyses of the metabolic intermediates in the pathways as well as the enzymes driving the bioprocesses. Targeted intracellular metabolite analyses were performed to monitor the different steps in the metabolic pathways. LTQ-Orbitrap identification strategies as well as triple quadrupole quantification strategies were applied. Methods for analysis of di-carboxylic acids and amphoteric metabolites were carried out using polarity switching. A label-free quantitative proteomics workflow was developed using the APEX tool (Lu. et al. 2007) for quantification based on spectral counting in combination with machine learning. This workflow was applied to verify the results of the genetic engineering and to monitor the cellular makeup at the protein level. The relative protein abundances of the enzymes involved in the production pathways were determined using selected reaction monitoring (SRM) methods. Both proteomics approaches were essential for confirmation of the genetic engineering process and lead generation for significantly improved production.

The obtained MS data on metabolites and proteins was combined with data generated by other techniques such as NMR and enzyme activity assays to generate leads to improve productivity even further. The dynamic interaction of expertises was essential for the successful development of succinic acid producing *S. cerevisiae* strains and 6-ACA producing *E. coli* and *C. glutamicum* strains.

PTu-162 Large Bio-particle Detection Technologies and Their Applications

13:30 – 14:40

Chung Hsuan Chen

Genomics Research Center/Academia Sinica, Taipei, Taiwan

Keywords:
Ion acceleration, biomolecular ion detection

Novel aspects:
（1）Developing synchronized pulsed acceleration to achieve a macro ion accelerator（2）Frequency scanning to trap cell/virus in the ion trap（3）Acoustic desorption of micro/nano particles

Abstract:
Recently, we have developed a few novel mass spectrometry technologies which include（1）single large biomolecular ion detector（2）biomolecular ion accelerator and（3）acoustic desorption micro-/nano- particle mass spectrometer.

It is well known that the detection efficiency is very low for charge amplification detector to measure very large biomolecular ions due to the low yield of the secondary electrons. We designed our detector based on the detection of secondary ions instead of secondary electrons to significantly enhance the detection efficiency. With this approach, a single large biomolecular ion such as IgM^+ was successfully detected. Due to the improvement in detection sensitivity, it can be quite valuable for top-down proteomic application.

Accelerator has been very broadly used for research in high energy and nuclear physics. During the past two decades, it has been extended to biomedical research and therapeutic applications. Nevertheless, only electron and atomic ion accelerators are available up to now. Few if any molecular ion accelerators have been reported. We developed the first macromolecular ion accelerator to accelerate very large molecular ions to reach to megavolt region. A series of well-timed pulsed voltages was used to accelerate very large macromolecular ions. With this approach, ions with mass-to-charge ratio (m/z) reaching 30,000,000 was successfully detected by a charge amplification detector such as a channeltron or an electromultiplier. We also coupled our macromolecular ion accelerator with a dual-ion trap mass spectrometer to make an accelerator-ion trap mass spectrometer for sensitive detections of very large biomolecules.

Up to now, all commercially available mass spectrometers have the limit on mass range. An ion with m/z above 1,000, 000 cannot be detected. We developed acoustic desorption charge monitoring mass spectrometer for the detection of bio-particles with m/z higher than 1,000,000. Laser induced acoustic desorption was used to desorb bioparticles from the sample plate into the ion trap. A frequency scanning ion trap was used to trap ions with very high m/z. The ion detection is based on charge detector. With this approach, we demonstrated particles with m/z as high as 10^{13} and mass at 10^{16} Da can be easily detected. We applied this mass spectrometer to measure microparticles, nanoparticles, cells and viruses. In addition, uptakes of various nanoparticles and virions by different cells have also been pursued. We found a large number of HIV virus is needed for an immune cell infection. This technology can also be used for quantitative drug measurement when nanoparticle is used as a drug carrier.

We are looking for further development of the above technologies for being able to do a single cell proteomics in the future.

PTu-163
11:10 – 12:20

Comparison between Single-cell and traditional MALDI-MS-based workflows for metabolome studies in Saccharomyces cerevisiae.

Alfredo J Ibanez[1], Stephan R Fagerer[1], Matthias Heinemann[2], Renato Zenobi[1]

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Keywords:
S.cerevisiae, 2-deoxy-D-glucose, Single-cell Metabolomics, Microarrays

Novel aspects:
For the first time, microarrays for mass spectrometry (a novel single-cell metabolomic platform) is used to study the non-genetic cell-to-cell heterogeneity observed within theoretically homogenous (i.e. clonal) yeast cell population.

Abstract:
Stochastic-induced phenotypic heterogeneity is one of the sources of cell-to-cell variation within a clonal cell population.[1] This non-genetic heterogeneity can only be studied using single-cell analytical platforms.[1-3]
Two years ago, our group introduced a novel analytical platform called microarrays for mass spectrometry (MAMS).[4] MAMS is capable of boosting the performance of commercial matrix-assisted laser desorption/ionization (MALDI) mass spectrometers in order to achieve single-cell sensitivity, when used as MALDI targets.
Here, we present the first in depth comparison between two MS-based metabolome approaches: (i) a population-level and (ii) single-cell level, for describing the biological response of a clonal cell culture of Saccharomyces cerevisiae (budding yeast) when applying the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG).
Our results show that the efficiency of 2-DG as a drug depends on the biological make-up of each individual cell within the isogenic cell culture. One potential explanation, observed using MAMS, for the cell-to-cell variation in the presence of 2-DG is the different levels of expression of glucose transporter proteins and/or glucose hexokinase enzymes in yeast cells.
Furthermore, we also present possible applications in which single-cell level metabolome studies, using the MAMS platform, provide a unique insight, such as: monitoring stochastic-induced phenotypic bistability.

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PTu-164
13:30 – 14:40

The Cuticular Structure and Lipid Composition of Arabidopsis thaliana Organs and Cells Determined by TOF-SIMS

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[1]Physical Electronics, Minnesota, USA, [2]University of British Columbia, British Columbia, Canada

Keywords:
botanical, lipids, imaging, TOF-SIMS, topography

Novel aspects:
We have employed TOF-SIMS chemical imaging to uniquely identify the distinct lipid composition of single cells comprising the stomates and trichomes on Arabidopsis organs.

Abstract:
The cuticle, or outer surface, of a plant has many functions including the regulation of growth, controlling the loss of water and the exchange of gases, and providing a protective barrier against many environmental stresses. The precise function of plant cuticles can best be understood on the basis of their characteristic chemical composition as well as their biosynthetic origin [1]. In the case of *Arabidopsis thaliana*, approximately 85 genes have been identified as responsible for the synthesis and metabolism of dermal and cuticular lipid chemistries [2]. The cuticle lipids are a mixture of acids, aldehydes and alcohols with chain lengths in the range of C_{24} - C_{32}. However, observing the cuticle chemistry of wild-type and knock-out varieties has met with challenges. Chief among these challenges concerns the sampling and isolation of cuticular wax components for chemical characterization. To date, characterization of the cuticular lipids has relied on manual dissection of the epidermis followed by solvation and chromatographic separation of the wax components. This sampling method reveals the average composition of the entire organ, but subtle inhomogeneities are overlooked. More importantly, specific differences in cellular chemistry are impossible to detect.

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is evolving as an important tool for 2D and 3D imaging mass spectrometry of biological specimens due to its unique capability to detect molecular and elemental ions with high sensitivity, sub-micron spatial resolution and without the sample treatments required by e.g. MALDI or fluorescence microscopy. For many biological specimens, the ability to garner reliable chemical distributions from samples having a large degree of surface topography is both desired and necessary ; to achieve this, an imaging mass spectrometer must have a large angular acceptance and a large depth-of-field. These analytical requirements are satisfied in the design of the PHI TRIFT V *nanoTOF*. The resulting elemental and molecular images provide important information regarding the composition of biointerfaces, tissues, and cells without the artifacts presented by topography and morphological variation.

The chemical imaging capability of TOF-SIMS is demonstrated by the characterization of epicuticular wax components present at the surfaces of *Arabidopsis thaliana* organs. High spatial resolution images obtained from intact organs and specialized cells illustrates the capability of TOF-SIMS to image and characterize biological chemistry at a spatial resolution of $< 0.3\ \mu$m. Total ion images and molecular ion images of cuticular lipids demonstrate the capability of the TRIFT analyzer to image organ surfaces without topographically induced artifacts. High mass spectra in both the positive and the negative secondary ion polarities reveal that the epicuticular surface of each *Arabidopsis thaliana* organ is comprised of distinct lipid compositions. Mass spectra acquired from specialized cells forming the stomates and trichomes reveal that, even for single cells on the surface of an organ, the lipid composition may vary. The differences in wax chemistry on each of the interrogated organs and cells of *Arabidopsis thaliana* will be presented and discussed. Structural assignments for characteristic lipid components comprising the epicuticular wax will also be presented.

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PTu-166
13:30 – 14:40

Proteomic Interrogation of MDSC Exosomes by Bottom-up, Middle-out and Top-down strategies

Catherine Fenselau[1], Meghan Burke[1], Waeowalee Choksawangarn[1], Avantika Dhabaria[1], Joseph Cannon[1], Suzanne Ostrand-Rosenberg[2]

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Keywords:
exosomes, tumor-micro-environment, proteomics, iTRAQ,

Novel aspects:
identification of > 2500 proteins in exosomes allows us to address their origin and their function.

Abstract:
Exosomes are membrane-bound extracellular vesicles that carry messages between cells. We hypothesize that the exosomes shed by myeloid-derived suppressor cells (MDSC) contribute to suppression of adaptive and innate immunity by communicating between MDSC, T-cells, natural killer cells, and macrophages in the tumor micro-environment. Pro-inflammatory agents increase the quantity and suppressive potency of MDSC, and thereby facilitate tumor progression in patients. We are studying the mechanisms by which inflammation drives MDSC in a mouse model. We have exploited bottom-up, middle-out and top-down proteomic strategies to inventory the proteins of exosomes associated with MDSC. In this presentation, relative and complementary merits of the three approaches will be discussed. We have also employed an iTRAQ strategy to quantitate differential protein abundances in exosomes from tumor- bearing mice with varying degrees of inflammation. Inflammation-related changes in exosomal proteins will be presented and compared with inflammation-related protein changes in the parent MDSC.

PTu-167

11:10 – 12:20

Identification of novel cytokinin degradation pathway during adventitious caulogenesis in Pinus pinea L. cotyledons with quadrupole-time of flight mass spectrometry

Ondrej Novak[2], Candela Cuesta[3], Karel Dolezal[1,2], Lucie Szucova[2], Lukas Spichal[2], Belén Fernández[3], Ana Rodríguez[3], Miroslav Strnad[2]

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Keywords:
cytokinin, identification, QqTOF

Novel aspects:
Novel cytokinin degradation pathway was identfied during adventitious caulogenesis in Pinus pinea L. cotyledons with quadrupole-time of flight mass spectrometry.

Abstract:

Background

Cytokinins (CKs) are a group of phytohormones which probably regulate the growth, development, and metabolism of all plants. The aromatic CK benzyladenine (BA) has been widely applied in *in vitro* culture for inducing shoot organogenesis. Our study of endogenous cytokinin profiles during the caulogenic process based on mature cotyledons of stone pine (*Pinus pinea* L.) showed a novel metabolic pathway of aromatic cytokinins based on modification of purine skeleton.

Methods

Three-year-old mature seeds from two half-sibling selected families and open-pollinated trees of *P. pinea* were used [1] and the samples were collected following the Alonso *et al.* [2] procedure. Extraction and purification of cytokinins was based on the method described by Novák et al. [3] , including modifications published later [4] . The samples were purified using a combination of a cation (SCX-cartridge) and anion [DEAE-Sephadex/C18-cartridge] exchangers. Combination of high performance liquid chromatography (HPLC) with quadrupole-time of flight mass spectrometry (QqTOF) was used for accurate and sensitive identification and qantification of cytokinins.

Results and Discussion

Using high-resolution MS, the naturally-occurring BA metabolites as well as new BA forms were identified. In comparision with previously publihed profiles of the BA metabolite pool [5,6] , the ribosyl and glycosyl forms were quantified as the most abundant metabolites. Moreover the biological activity of identified BA and its derivatives were compared in various CK bioassays. The results indicate that BA uptake during the caulogenic process may be possible to regulate not only by known cytokinin pathways. Finally, the feeding experiment with stable isotope-labelled standard, $^{15}N_4$-BA, confirmed our indetification of the novel metabolic CK pathway.

Conclusions

The identification of the novel BA forms demonstrates that the novel cytokinin pathway is used as a control mechanism of BA uptake from the bud induction medium during adventitious caulogenesis in cotyledons of *P. pinea*. Our results help to understand the processes associated with embryo germination in plant tissue culture.

Acknowledgements

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PTu-168
13:30 – 14:40

Activation Kinetics and Dimerization of STAT5 Proteins Followed by Quantitative Mass Spectrometry

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Keywords:
STAT 5 Isoforms, dimerization, phosphorylation, immunoprecipitation, relative quantification

Novel aspects:
Recognition of the connection between immunoprecipitation by isoform-specific STAT 5 A/B antibodies, phosphorylation-induced STAT 5 dimerization and STAT 5 phosphorylation degree determination.

Abstract:
The mouse genome contains one gene each for STAT 5 A and STAT 5 B on chromosome 11. Due to possible functional differences between both STAT 5 isoforms, individual analysis of their abundance, activation and dimerization is of interest. The isoforms A and B of mouse STAT 5 differ in about 9 % of their amino acidpositions, enabling their isoform-specific analysis by antibodies and mass spectrometry. The variety of single amino acid substitutions generates multiple closely related tryptic peptides for each isoform. Comparing signal intensities of these similar but unique peptides allows label-free relative quantification of both isoforms. One such variation (STAT 5 A V691/STAT 5 B A696) allows A- and B-specific phosphorylation analysis of the STAT 5 activation motif located atTyr694 in STAT 5 A and at Tyr699 for STAT 5 B. Analysis of the dynamics of STAT 5 activation by Epo in the mouse cell line BaF 3 -EpoR via a combination of immuno precipitation and mass spectrometry revealed a complex interplay between the isoform-specific degree of phosphorylation at the activation motif and relative A/B isoform abundances. This observation was made possible on the basis of accurate data for the degree of phosphorylation at the activation motif, which were generated by the use of one-source peptide/phosphopeptide standard pairs and LC-MS. Dimerization of phospho-STAT 5 was hypothesized as the main source of this phenomenon.

To check this hypothesis, additional STAT 5 activation experiments by Epo in BaF 3 -EpoR cells were performed and analyzed using immunoprecipitation by different STAT 5 antibodies with established STAT 5 A/B specificity. Their isoform-selectivity was further verified by mass spectrometric sequencing of their corresponding blocking peptides. These experiments revealed that (i) A and B isoforms of STAT 5 occur at a ratio of 2 : 1, that (ii) both STAT 5 A and STAT 5 B show a highly similar degree of phosphorylation, and that (iii) phospho-STAT 5 dimers are formed mainly without isoform selectivity.

PTu-169
11:10 – 12:20

Lapatinib-resistant gastric cancer cells express cancer stem cell-like cell surface antigens

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Keywords:
Cancer stem cell, Lapatinib-resistant, Cell surface antigens

Novel aspects:
Global profiling of unique or highly expressed cell surface antigens of cancer stem cell (CSC) -like cells

Abstract:
Lapatinib is a dual inhibitor for HER 2 and epidermal growth factor receptor tyrosine kinases that has shown promising in vitro results in inhibiting the growth of HER 2 (+) cancer cells. However, some tumors either do not respond or develop resistance to the agent. To evaluate the properties of drug-resistant cells, we isolated a lapatinib-resistant, HER- 2 positive gastric cancer cell population by incubating lapatinib-sensitive SNU-216 cells for a prolonged period with lapatinib. The drug-resistant cells were characterized using a panel of cell surface markers identified from a cancer stem cell (CSC) -like cell population derived from an Oct 3 / 4 -controlled MB-453 breast cancer cell line. The panel of cell surface markers was identified using a large-scale proteomic analysis and included the known CSC markers CD44, CD133, EpCAM, and CD151 as well as newly identified CSC-specific cell surface antigens. Immunoblot analysis of lapatinib-resistant SNU-216 gastric cells showed that both the known CSC markers and the newly identified CSC cell surface antigens were significantly upregulated compared to lapatinib-sensitive SNU-216 gastric cells. These results suggest that the development of resistance to lapatinib is associated with a shift towards a CSC phenotype.

PTu-170 Tissue specific profiling of the Arabidopsis thaliana auxin metabolome

13:30 – 14:40

Ondrej Novak[1,2], Eva Henykova[1,2], Ilkka Sairanen[2], Mariusz Kowalczyk[2], Tomas Pospisil[3], Karin Ljung[2]

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Keywords:
IAA, auxin, metabolite profiling, Arabidopsis

Novel aspects:
A new sensitive and selective method for multiplex quantification of IAA and its precursors and degradation products in Arabidopsis tissues.

Abstract:
Hormonal profiling methods use modern analytical tools based on fast chromatographic separation and mass spectrometric quantitative analysis. The plant hormone auxin is believed to influence almost every aspect of plant growth and development. We have developed and validated a method for profiling the majority of known auxin precursors and conjugates/catabolites in small amounts of Arabidopsis tissue. Our method includes trace analysis of 21 compounds with different polarity, acidity and basicity as well as stability and abundance in crude plant extracts. We found that a polymer based (Oasis HLB) sorbent was the best tool for one-step purification, including a new derivatization method to quantify the most labile of the auxin precursors. The process was completed by a single chromatographic analysis of auxin metabolites in 12 minutes using an analytical column packed with sub-2-microne particles. In multiple reaction monitoring mode, the detection limit for most of the analytes ranged from 1.0 to 5.0 fmol and the achieved linear range was at least five orders of magnitude. Finally, we profiled the auxin metabolome in root and shoot tissues from different Arabidopsis thaliana ecotypes and auxin overproducing mutant lines, showing substantial differences in the metabolite pattern between the lines and between different tissues. We also observed differences in abundance of several orders of magnitude between different auxin metabolites, indicating the relative importance of different auxin precursors and conjugates/catabolites for maintaining auxin homeostasis. We now have a powerful tool to get a better understanding of the regulation of auxin metabolism during plant development.

PTu-171
11:10 – 12:20

In Situ Pressure Probe Picoliter Single-Cell Sap Sampling and Mass Spectrometry Metabolite Profiling of Living Plants

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Keywords:
Intact cell, quantitation, metabolomics, glycomics,

Novel aspects:
Mass spectrometry analysis of picoliter single-cell sap sampled by using the pressure probe is performed. Single-cell sap volume sucked is controlled and measured. Additional intact-cell properties can be evaluated.

Abstract:

In plants, anatomy of different organs and tissues is not necessarily uniform and cells located at different tissues and depth may vary significantly in function, morphology and metabolite composition. Therefore, single-cell metabolite profiling to be applied to plant science demands for accurate and fully-managed sampling from different tissues and cell layers. The sampling is more challenging due to the difficulty in managing femto- to nanoliter volume of sap each plant cell can provide. Additionally, the single-cell sap volume must be measured if natural changes in the abundance of metabolites of cells are to be analyzed. In our model plant, tulip, parenchyma cells located at inner layers of bulb scale tissue (e.g. 400 μm from the surface of cuticle) contain abundant starch, long-chained fructans, underivatized soluble sugars and amino acids. They function as the source of nitrogen and carbon for growth of new flower stalk. For flowering, bulbs need to be stored at low temperatures (4-10°C) for about two months. We targeted those parenchyma cells in cold-stored bulbs.

We used a cell pressure probe for in situ single-cell sampling followed by shotgun metabolite profiling with UV-MALDI TOF MS (AB SCIEX 5800 system) and nanoESI MS (ThermoFisher Exactive Orbitrap™ mass spectrometer). The quartz capillary of the pressure probe was filled with silicon oil and air-tightly connected to a pressure transducer. Pressure inside capillary is manipulated with a rod moved back and forth by a micrometer. Therefore, while the capillary was penetrating to a pre-determined tissue depth, the sap of cells located on the way to the target cell was prevented from entering the capillary tip by regulating the oil pressure. The movement of capillary and penetration depth was controlled and measured with a piezo-manipulator and with previous anatomy of tulip bulb tissues, depth of penetration to access favorite cells became possible. After penetration of the capillary into a cell, cell sap enters the capillary tip due to turgor pressure naturally exists in living plant cells ; and a meniscus forms at the interface of the oil and analyte solution inside the capillary. The diameter of parenchyma cells in tulip bulb scale varies in the range of 50-150 um and we obtained 100-600 pL cell sap from each cell. The tip with cell sap sample inside was photographed and the volume of the picoliter sample was accurately measured.

For UV-MALDI MS, the picoliter cell sample was injected to a 1 μL water droplet hanging from a pipette tip. The picoliter cell sample (in 1 μL water droplet) was deposited on previously dried matrix spots on stainless steel MALDI plates. We have examined several matrixes for single-cell metabolite profiling and in this study we used 2,4,6-trihydroxyacetophenone (THAP), 2,5-dihydroxybenzoic acid (DHB) and titanium silicon oxide (SiO_2) (TiO_2) nanoparticles. With (SiO_2) (TiO_2) nanoparticles good linearity of signal abundance vs. number of picomoles of standard compounds was yielded. With all matrixes, signals of cell sap metabolites were detected. Particularly, big neutral oligosaccharides could be easily analyzed with UV-MALDI MS. For nanoESI MS analyses, cell sap sample was transferred to a 5 μL water droplet and then injected into the electrospray ion source. NanoESI MS was also successful in metabolite profiling of single-cell sample ; however, smaller oligosaccharides could be detected. In addition to sugars, amino acids, organic acids, tuliposide, putrescine, and GABA could be detected with both MALDI and nanoESI MS. Relative abundance of sucrose and kestose in parenchyma cells located at the depth of 100-500 μm was determined. The limit of detection of sucrose was similar (2-5 pmol) in both techniques but dynamic range of detection was wider in MALDI MS (with 2-25000 pmol) than nanoESI MS (5-2500 pmol).

PTu-172
13:30 – 14:40

In-situ Analysis of Plant Bioactive Molecules by Live Single-cell Mass Spectrometry

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Keywords:
Plant, Bioactive Substances, Live Single-cell MS, Photosynthesis

Novel aspects:
Light responding metabolism of glucosinolates in a live plant was directly analyzed at single cell level.

Abstract:

Introduction

Secondary metabolites in plants have been attracted attention by their bioactive property such as antioxidant and antibacterial effects. Biosynthesis of these compounds is affected by plant's growing condition. LC-MS studies of plant metabolites have been reported in which large numbers of cells were homogenized to get averaged data. These processes are time-consuming, and molecules which are volatile such as IsoThioCyanates (ITCs) or unstable molecules such as glucosinolates, can be lost during preparation. We applied in-situ Live Single-cell MS method which allows pretreatment-free analysis for detection and identification of molecules in a certain region of a plant tissue. In this study, the diversity of glucosinolates in plants with different growth conditions is reported.

Methods

We left radish sprouts in the dark for five days post seeding, and exposed to white or red light for further two days. Then we observed pith and cortical tissues in a cross-sectional slice of a radish sprout's stem by a stereomicroscope. A representative cell in every tissue was selected, and cellular content was micro-sucked into a nanospray tip (HUMANIX). Then, ionization solvent (acetonitrile) was added into the needle followed by mass spectrometric analysis with a LTQ-Orbitrap Velos (Thermo Fisher Scientific) equipped with nanoESI source at both positive and negative ion mode. At the same time, SIM (selected ion monitoring) scan mode was used for high-sensitive analysis. Spray voltage was around 1000 V (positive mode) or -800 V (negative mode). Detected peaks were identified by MS/MS and exact mass measurement.

Results

At positive ion mode, we detected several ITCs such as 4-methylthio-3-butenyl-isothiocyanate (m/z 160.0249), isopropyl isothiocyanate (m/z 102.0372) in a cell on a cross-sectional slice of radish sprout stem. Besides, we used negative ion mode to detect several glucosinolates such as 4-methylthio-3-butenyl-glucosinolate (m/z418.0295), glucobrassicin (m/z 447.0527), glucoerucin (m/z 420.0451). This method enabled us to perform direct and quick analysis of ITCs and glucosinolates which are highly reactive and easy to be degraded during pretreatment for LC-MS. In addition, we could identify restricted localization of these compounds. Glucosinolates were detected only in a cell on cortical tissue, whereas ITCs were detected mainly in pith. Their localization was usually lost in a conventional LC-MS. Then, we performed spatio-temporal analysis of these bioactive compounds with illuminated radish sprouts. Under white light exposure, levels of most glucosinolates were higher than those in the condition of red light exposure. On the other hand, level of glucobrassicin was exceptionally higher under red light exposure, whereas its intermediates showed different distributions on a cross-sectional slice of radish sprout stem. Among them distribution of indole-3-acetaldehydeoxime, precursor of glucobrassicin, was quite different. It was detected mainly in pith under white light exposure, but in cortical tissue under red light exposure. Consequently, these results showed this method has enabled us to detect their bioactive substances and analyze quantitative variations of molecular distribution in a plant tissue easily and quickly. More detailed studies of light responding metabolism are under progress.

PTu-174
13:30 – 14:40

2D-DIGE analysis of the role of S-nitrosoglutathione reductase in lipopolysaccharide-challenged mice

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Keywords:
Oxidative stress, 2 D-DIGE, Endoplasmic reticulum, Cancer, Liver proteins

Novel aspects:
Deficiency of S-nitrosoglutathione reductase compromises expression of the ER stress-related proteins in LPS-treated liver.

Abstract:
Introduction: *S*-Nitrosoglutathione reductase (GSNOR) is a key regulator of protein *S*-nitrosylation, the covalent modification of cysteine residues by nitric oxide that can affect activities of many proteins. We recently discovered that excessive *S*-nitrosylation from GSNOR deficiency in mice under inflammation inactivates the key DNA repair protein O^6-alkylguanine-DNA alkyltransferase and promotes both spontaneous and carcinogen-induced hepatocellular carcinoma. To explore further the mechanism of tumorigenesis due to GSNOR deficiency, we compared the protein expression profiles in the livers of wild-type and GSNOR-deficient ($GSNOR^{-/-}$) mice that were challenged with lipopolysaccharide (LPS) to induce inflammation and expression of inducible nitric oxide synthase (iNOS) .
Methods: We performed two dimensional fluorescence difference gel electrophoresis (2 D-DIGE) to identify differentially expressed proteins in the liver tissues of wild-type and $GSNOR^{-/-}$ mice following LPS-treatment. To identify the differentially expressed proteins by mass spectrometry (MS) and database search, the spots were destained, reduced with dithiothreitol, alkylated with iodoacetamide and then digested overnight with trypsin. Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) MS and MS/MS analyses were performed using the AXIMA-Performance mass spectrometer (Shimadzu) . alpha-Cyano- 4 -hydroxycinnamic acid was used as the matrix.
Results and Discussion: 2 D-DIGE analysis identified 38 protein spots of significantly increased intensity and 31 protein spots of significantly decreased intensity in the $GSNOR^{-/-}$ mice compared to those in the wild-type mice. We subsequently identified 19 up-regulated and 19 down-regulated proteins in $GSNOR^{-/-}$ mice using MALDI-TOF MS and MS/MS. Immunoblot analysis confirmed in $GSNOR^{-/-}$ mice a large increase in the expression of the pro-inflammatory mediator, S100A9, a protein previously implicated in human liver carcinogenesis. We also found a decrease in the expression of multiple members of the protein disulfide-isomerase (PDI) family and an alteration in the expression pattern of the endoplasmic reticulum (ER) chaperones in $GSNOR^{-/-}$ mice. Furthermore, altered expression of these proteins from GSNOR deficiency was prevented in mice lacking both GSNOR and iNOS. In addition we detected *S*-nitrosylation of two members of the PDI protein family.
Conclusions: These results suggest that *S*-nitrosylation resulting from GSNOR deficiency may promote carcinogenesis under inflammatory conditions in part through the disruption of inflammatory and ER stress responses.

PTu-175 Proteomic analysis of low temperature resistant system in bacteria

11:10 – 12:20

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Keywords:
Bacteria, cold shock resistant system, nano LC-MS/MS

Novel aspects:
This proteomic approach is shown to be ideal as a primary analysis for examination of various physiological characteristics in bacterial cells comparably to a DNA array analysis approach.

Abstract:
Under the cold condition, the increased production of cold shock proteins (CSPs) is mainly a consequence of the increased stability and preferential translation of the corresponding mRNAs. This response, which in general terms in similar in different mesophilic bacteria, helps to counteract a number of stresses derived from low temperatures such as reduced membrane fluidity, reduced enzyme activity, and the greater stability of DNA and RNA secondary structure that can diminish the efficiency of replication, transcription and translation. The bacteria stay long time under low temperature, the growth of cell was stopped. After stopped growth, temperature returned 4 °C to 37°C, the cells be able to grow normally. Usually, the knowledge of cold shock response system was obtained from model-bacteria (*Escherichia coli* and *Bacillus subtilis*) and 15°C. But most model-bacteria cannot grow at under 16°C. Most studies on the biology of bacteria have been performed on cells grown at 30°C, its optimum growth temperature. However, when growing in natural habitats, bacteria frequently live much low temperature place that appears under 10°C and occupies 70% of the earth. *Pseudomonas putida* F 1 is model bacteria living in soil, this family was isolated from the iceberg of the south antarctic pole. Because, we think that the genome of this strain was included essential genes of constant low temperature growth.

The cold shock system is suggested that it is the stress response protein to rescue from inconvenient environment of low temperature, and the possibility that there is not the protein necessary to normal grow under the constant low temperature. This present works report proteomic analysis comparing two conditions, the one is 30°C, 16°C and 4 °C, and the second is cold shock stress phase (CSSP) and constant low temperature phase (CLTP) .

To obtain the low temperature resistant system of bacteria, we prepared three kind of the temperature, 30°C is optimum growth temperature, 4 °C is low temperature that almost model-bacteria cannot grow under this condition, 16°C is intermediate temperature previous two conditions. *P. putida* F 1 was cultured at its optimum growth temperature in minimal salt medium. After grew at exponential phase (O.D.600 = 0.3) , all proteins were extracted from cell. The proteins were separated by SDS-PAGE, and gels were cut into about 60 slices for in-gel digestions by trypsin. Each peptide mixture was analyzed by nano LC-MS/MS (Thermo Fisher Scientific, USA) for protein identification. The LC-MS/MS data were searched by the *P. putida* F 1 database in NCBI, and, expression level of each gene were appeared by emPAI value calculating from Mascot program (ver. 2.3.01) .

About 2000 proteins were identified based on more than two unique peptides. The number of proteins expressed under several temperatures was about 40 % of all proteins (5250) . The overview of expression genes was appeared to compare protein expression at 30°C. In 16°C, a group of 340 genes displayed a two-fold significant increase at CSSP, and 789 genes displayed a two-fold increase under the phase of the CLTP. In 4 °C, the numbers of CSSP genes are 330, and CLTP genes are 1051. The interesting result in this, almost genes that showed expression more than two-fold were phase-specific. The expression increase genes were separated about function, we don't find the common genes of low temperature (CSSP vs CLTP and 16°C vs 4 °C) . When sigma factor (RNA polymerase subunit) expression was checked in several conditions, that expression pattern was independently about each phase. RpoS (the homolog gene of *E. coli* control CSPs expression) was only induced in CLTP at both temperatures. This result shows that the mechanism of low temperature resistance is different between the CSSP and the CLTP, 16°C and 4 °C.

PTu-176
13:30 – 14:40

IgSF11 is a member of the immunoglobulin superfamily that promotes both neuronal adhesion and cell proliferation

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Keywords:
immunoglobulin super family (IgSF) , neuronal adhesion, cell proliferation,LC-MS/MS

Novel aspects:
IgSF11 is one of cell adhesion molecules ubiquitously expressed in the CNS, however its function remains to be clarified. Here we present a piece of data to show its functions.

Abstract:
IgSF11 is a member of the immunoglobulin super family (IgSF) that possesses two immunoglobulin-domains, and is expressed ubiquitously in the mammalian central nervous system (CNS) . IgSF11 is up-regulated in gastrointestinal and hepatocellular carcinomas, and its down regulation leads to lower grades of malignancy. In the CNS, while the molecule is regarded as a cell adhesion molecule as judged from its structure, it is still unclear whether IgSF11 functions in the neuronal processes of development and maturation.

To assess this possibility, we first examined the effects of IgSF11 on the morphological differentiation of cultured neurons. When culture dishes were pre-coated with mutant IgSF11 proteins consisting of the extracellular domain, hippocampal neurons prepared from mouse embryos formed cell aggregates within a few days of being cultured, and their neurites were observed to fasciculate through the homophilic interaction of IgSF11. Enhanced IgSF11 expression by gene transfer brought similar changes in cultured neurons. In contrast, a couple of short hairpin RNAs (shRNAs) complementary to IgSF11 showed neurite branching as a result of the silencing of IgSF11 gene. Furthermore, IgSF11 proteins with various deletions of the cytoplasmic domain also stimulated such branching, suggesting that either the PDZ (PSD-95/D 1 h/ZO 1) binding region or some unknown regions in the cytoplasmic domain are requisite sequences needed to regulate both the neurite fasciculation and cell aggregation induced by IgSF11. Among the kinase inhibitors we tested, only those inhibitors for Protein Kinase C (PKC) attenuated both branching and cell adhesion.

We next analyzed whether or not IgSF11 affects neuronal differentiation of mouse embryonic carcinoma (P19) cells inducible by retinoic acid treatment. Differentiated neuronal P19 cells showed lowered expression of IgSF11. As expected, forced expression of IgSF11 led to a decrease in the number of cells undergoing neuronal differentiation, instead allowing cells to proliferate in an undifferentiated state. A couple of shRNAs, that effectively down-regulate the IgSF11 gene, cause an increase in the numbers of both neurons and glial cells, indicating that IgSF11 affects multiple processes during neuronal differentiation. cDNAs encoding mutant IgSF11, that were devoid of the PDF binding region, failed to stimulate proliferation of undifferentiated P19 cells. Interestingly, inhibitors of PKC attenuated IgSF11-induced cell proliferation as well. Therefore, we conclude that there may exist molecules that interact with IgSF11, or are affected by transduction of IgSF11 signal, that are involved in the neuronal differentiation of P19 cells and morphological changes in cultured hippocampal neurons.

To search for molecules that interact with IgSF11,we constructed and isolated cell lines stably expressing IgSF11. We prepared cell extracts after undergoing isotopic protein labeling of the cell lines, immunoprecipitated IgSF11 with its binding proteins from the cell extracts, or prepared cell extracts with other methods in order to analyze molecules using LC-MS/MS combined other methods. As a couple of candidates have been detected to date, we have been extensively examining these to see if they satisfy criteria as molecules with which IgSF11 exerts its functions.

Although IgSF11 is a small member of the IgSF ubiquitously expressed in the CNS, these data, taken together, strongly suggest that its expression affects the development and morphological changes of neurons within the CNS, and that there are some molecules that interact with IgSF11 or mediate its signal transduction.

PTu-177
11:10 – 12:20

Live Single-cell Mass Spectrometry for Tracing Metabolic Processes in Organelle

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Keywords:
Single cell, Organelle, Isotope labeling, Metabolomics

Novel aspects:
Metabolic pathways even in a live single cell organelle was clarified by the Live Single-cell Mass Spectrometry.

Abstract:
Introduction
We have developed a direct mass spectrometric method called "Live Single-cell Mass Spectrometry. " This method enables us rapid metabolic analysis in the visualized organelle even in a live single cell. This method analyzes metabolites and its metabolic pathways even in an organelle with its behavior and sub-cellular localizations. Furthermore, using stable isotope labeled reagents we can trace the metabolic processes in a live single cell.
In this study, we applied this method to analyze the localizations of the histidine and histamine metabolites and to trace their metabolic processes in a live single rat basophilic leukemia (RBL-2H3) cell.

Methods
RBL-2H3 cells were cultured in DMEM and incubated at 37° C in CO_2 incubator. A cell contents (secretory granule or cytoplasm) of a live RBL-2H3 cell aimed under view by CCD video camera mounted on an inverted microscope, was micro-sucked into a nanospray tip (Cellomics Tip, HUMANIX, Japan) with a micro-manipulator. Ionization solvent containing 0.5 % formic acid (for positive mode) was added to the content in a nanospray tip. Mass spectrometric detection was performed by a LTQ Orbitrap XL mass spectrometer equipped with nano-ESI ion source. Spray voltage was around 1000 V (for positive mode) . To increase sensitivity of peak detection, we used the SIM mode for single-cell molecular detection. For tracing the histidine and histamine metabolic reactions, RBL-2H3 cells were cultured ^{15}N labeled DMEM which histidine was replaced to ^{15}N isotope labeled histidine.

Results and Discussion
In order to clarify micro-sucked granule contents specifically, we used di-protonated Quinacrine ion peak (*m/z* 200.6111) as a marker ion peak of granule-sucked sample. The histidine and histamine metabolites ion peaks were detected in a granule and/or cytoplasm in a single RBL-2H3 cell. A statistic analysis of t-test was performed for these peaks to clarify the sub-cellular localization between a granule and cytoplasm (more than 5 cell samples for each) . The ion peak at *m/z* 156.0764 was detected in granule side and identified as histidine by exact mass number (within 3 ppm error) and MS/MS fragment pattern. Histamine (*m/z* 112.0868) , one of the histidine metabolites was found in granule specifically. The other histidine metabolites (e.g. imidazole acetaldehyde (*m/z* 111.0552) , methylhistamine (*m/z* 126.1026) , urocanic acid (*m/z* 139.0501) , 1-methylhistidine (*m/z* 170.0921) , and so on) were also found in granule side. The results show that histidine and histamine metabolic pathways existing in a granule. From ^{15}N isotope labeled histidine ($C_6H_9{}^{15}N_3O_2$) treated RBL-2H3 cell, ^{15}N labeled histidine (*m/z* 159.0678) and histamine (*m/z* 115.0779) , and its metabolites, imidazole acetaldehyde (*m/z* 113.0493) , urocanic acid (*m/z* 141.0443) and so on were detected in granule samples. From these results, it was clarified that histidine transport into granule and clarified the histidine metabolic pathways locate in granule.
This method has enabled us to analyze not only location of metabolic pathways but also metabolic processes of interest molecules and it can be used in various organelle metabolomics in a live single cell.

PTu-178
13:30 – 14:40

Development of Micro-area-specific Extracting Analysis for Molecules in Tissue Section

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Keywords:
direct tissue analysis, micro solvent extraction, nanoESI, micro-area-specific

Novel aspects:
Small molecules exist in specific micro-area of tissue section were analyzed by micro solvent extraction using nano-spray tip.

Abstract:

Introduction

Animal tissue is composed of some aggregations of differentiated cells, and each group play a role and maintain whole function of the tissue. The local existence of molecules in particular part of tissue is important in order to understand system of tissue, or effects of drug. The location of molecules has been found by technology of MS imaging such as MALDI or SIMS imaging. However, these methods have problems that small molecules or molecules inside of cell is hardly detected. In this study, new methods by which, molecules exited in specific area of about 10 μm wide in a frozen tissue section is selectively extracted, trapped and measured by mass spectrometry, was developed by applying Live Single-cell MS method.

Methods

A piece of chicken liver was frozen in hexane cooled in liquid nitrogen, and was embedded in Frozen Section Compound. It was sliced into 10 μm thick of tissue sections at -20&#8451 ; using freezing microtome (Leica CM3500S, Leica Micro Systems) . Sections were mounted on glasses and dried in room temperature. Head of nanospray tip (Cellomics Tip, HUMANIX) including 1 μL of solution for extracting (100%MeOH, 50%MeOH, 100%H_2O, 100%Acetone) was adjusted to area close to or distant from vessel using the micro manipulator, And solvent was taken in and out from the tip with pressure in order to extract the molecules under microscope observation. The tip was added 3 μL of solvent (90%MeOH/9.0%H_2O/1.0%formic acid) behind and directly ionized and detected using nanoESI-MS.

Result and Discussion

Solvent taken in and out of nanospray tip covered circular area of about 10 μm wide on liver tissue section. 1.0kV of Ionspray Voltage was directly added the nanospray tip trapping the extraction of tissue and molecules in liver was directly analyzed by LTQ Orbitrap Velos (Thermo Fisher Scientific) , at positive mode, and by full scan at a range of m/z 70-2000. Although solvent used for extraction was 100%MeOH, 50%MeOH, 100%H_2O or 100%Acetone, most peaks, including small molecules such as serine (*m/z* 106.049) , cytidine (*m/z* 244.093) , creatine (*m/z* 132.076) and fatty acids, phospholipids, were detected, when 100%MeOH was used. When acetone was used, lipids, which have more than 1000 molecular weight tended to be detected higher than when other solvent were used. Mass spectrum data was aligned by MarkerView Software (AB SCIEX) and t-test (peaks detected in area close to vessel vs. distant from vessel) was performed. In the result, 6 peaks were specific in close to vessel, and 47 peaks were specific in distant from vessel ($p>0.01$) . Identification of these peaks using MS/MS analysis was performed and a fatty acid, which has the formula of $C_{18}H_{30}O_2$, is specific to the area distant from vessel. It is possible that activity of metabolism or transport of the lipid vary at a different site of the liver. Analysis of the related metabolites to this lipid and identification of specific peaks in each site are under progress.

PTu-179
11:10 – 12:20

In-situ Analysis of Phototropic Molecules in plant by Live Singe-cell Mass Spectrometry

TAKASHI FUJII[1], SACHIKO DATE[2], HAJIME MIZUNO[1], NAOHIRO TSUYAMA[1], TSUTOMU MASUJIMA[1,2]

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Keywords:
live single-cell mass spectrometry, plant metabolite, phototropism

Novel aspects:
Localized metabolites by light exposure was detected using in-situ live single-cell MS

Abstract:

Introduction

Plants have unique characteristics called phototropism that they can curve their stems toward light source to get more light energy for their growth. It has been said that increase of intracellular concentration of auxin, a plant hormone related in plant growth and body development, is involved in this phototropism process. However, molecular mechanisms of phototropism from the view point of low molecular weight metabolite have been remained unclear. In order to clarify its spatio-temporal molecular mechanism, it is important to analyze metabolites quantitatively and qualitatively. In this study, we performed live single-cell mass spectrometry (MS) using radish sprouts to identify the molecules correlated with phototropic reaction by exposing light.

Methods

Radish sprouts were grown in the humidified dark room at 24 ± 1 degrees C for 5 days. Subsequently, some of them were irradiated with blue light from horizontal direction for 0, 8, 16, 24 hours respectively. Upper part of irradiated sprout stems bent toward light source whereas lower part of stems kept straight. Then, the stems were cut in longitudinal direction. Surface cells in 4 parts (bent lighted side, bent shaded side, straight lighted side and straight shaded side) were targeted, and their cellular contents were micro-sucked into nanospray tips (HUMANIX, Japan) under a stereomicroscope. Mass spectrometric detection was performed by a LTQ Orbitrap Velos Pro (Thermo Fischer Scientific, USA) equipped with nano-ESI source at both positive and negative ion mode.

Results

Principal component analysis (PCA) on spectral data of stem parts, bent shaded side and lower shaded side, showed similar trend of spectral pattern because they were located adjacent to each other in 2D score plot. So, we analyzed difference of molecular distribution between lighted side and shaded side in detail. Glucose ($[M\text{-}H]^-$, m/z 179.0561), source of nutrition, glutamine ($[M\text{-}H]^-$, m/z 145.0618) and asparagine ($[M\text{-}H]^-$,m/z 131.0462), source of nitrogen, were increased in the lighted side in comparison with no light exposed shaded side. These results show that those increased molecules were produced by photo-synthesis in the lighted side, and in the shaded side those were consumed by the growth without novel production. It is possible that the concentration of metabolites were attenuated by cell elongation in shaded side. In addition, a few dozens of metabolites showed limited existence. Among them, glucosephosphate ($[M\text{-}H]^-$, m/z 259.0224) showed unique trend : this metabolite was higher in the lighted side than in shaded side after light exposure, but surprisingly it was much higher before exposure. Now, further investigation of molecular mechanism based on these results, and comprehensive analysis of metabolites involved in phototropism are under progress.

PTu-180 Analysis of Specific Molecules in Allergic Granule by Live Single-cell Mass Spectrometry

13:30 – 14:40

YUKI YAMAMOTO[1], SACHIKO DATE[2], HAJIME MIZUNO[1], NAOHIRO TSUYAMA[1], TSUTOMU MASUJIMA[1,2]

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Keywords:
live single-cell mass spectrometry, RBL-2H3 cells, granule, orbitrap mass spectrometer

Novel aspects:
By Live Single-cell MS method, specific molecules in a granule and their metabolism were directly clarified.

Abstract:
<Introduction>
Intracellular molecules are distributed in sub-cellular organelle as a function of each, and they control vital response by changing their amount or localization. In allergic response, mast cells secret molecules in granule and then allergic symptoms are triggered. So, analysis of molecules in granule gives one of the important information to understand the allergic mechanism. In order to clarify allergy-inducing molecules, we analyzed cytoplasm and allergic granules in RBL-2H3 cell line by using the "Live Single-cell Mass Spectrometry ". This method can trap particular region in a single cell under a video-microscope, and detect molecules directly by mass spectrometer. Comparing peaks of cytoplasm to those of allergic granule, we analyzed specific molecules of each organelle.

<Method>
RBL-2H3 cells were observed under an inverted video microscope, then granule or cytoplasm was trapped into a nanospray tip (Cellomics tip, HUMANIX, Japan) using a micro manipulator. After sampling, ionization solvent (80%methanol, 1%formic acid) was added to the nanospray tip, and then high voltage was applied to the tip for generating charged spray droplet. Mass spectrometric detection was performed by a LTQ Orbitrap (Thermo Fisher Scientific) equipped with nano ESI source. We optimized measurement condition and SIM (selected ion monitoring) scan mode for highly sensitive analysis. Paired t-test for extracting specific peaks of granules was performed. Molecular formation of detected ion was identified by exact mass number.

<Results>
We used quinacirne ion peak (*m/z* 200.6111) as the marker to confirm the successive granule suction in a RBL-2H3, because accumulation of quinacrine into granules was observed by fluorescence microscope. By comparing peaks of granules to those of cytoplasm, over 50 specific peaks of granule were detected at positive ion mode, such as creatinine (*m/z* 114.0663) , metabolites of arginine, and 4-hydroxyphenylacetate (*m/z* 153.0544) , metabolites of tyrosine, and so on. In addition, histamine (*m/z* 112.0869) and metabolites of histamine such as methylimidazole acetaldehyde (*m/z* 125.071) and methylimidazole acetic acid (*m/z* 141.066) were also detected. These results indicated that histamine metabolic pathway exists in granule. On the other hand, taurine (*m/z* 148.0039) and tryptophan metabolites such as formylkynurenine (*m/z* 237.0873) , hydroxyindoleacetate (*m/z* 192.0655) were detected from cytoplasm specifically. Identification of other candidate molecules by MS/MS analysis is under progress. In addition, we applied stimulus to RBL-2H3 by calcium ionophore A23187. As a consequence, the intensity of histamine metabolites decreased by administration of A23187, whereas the intensity of histamine itself stayed about the same. This result suggest that metabolism of histamine is suppressed by the stimulus.

PTu-181
11:10 – 12:20

Time Dependent Changes of Intracellular-Signal-Relating Small Compounds in Allergy Model Cell Line, RBL-2H3

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Keywords:
Orbitrap, Allergy, RBL-2H3, Intercellular-Signaling

Novel aspects:
Stimulated-cell-specific compounds and their time dependent changes were clarified in an allergy model cell line, RBL-2H3.

Abstract:
PURPOSES
There are enormous small compounds and their behavior in an intracellular signaling remains unclear, while large compound such as protein participated have been studied in detail. However small compounds are closely related to an intracellular signaling. Therefore it is important to investigate their intracellular localization and time dependent changes of small compounds in clarifying the perspective of signaling. In this study, using the Rat basophilic leukemia (RBL-2H3) cells as an allergy model cell line, we compared small compounds between in stimulated and not stimulated cells and analyzed time dependent changes after stimulation in the signaling of allergic reaction.

METHODS
Rat basophilic leukemia (RBL-2H3) cells were stimulated with 1μM calcium ionophore A23187 and extracted by methanol for 5, 10 and 30 minutes. Cell extracts were applied a LC-MS. We used L-column 2 C8 (1.5 x 150 mm, 5μM, CERI) connected to LC (LC-20AD, SHIMADZU). The mobile phase was 10mM ammonium formate in 90% methanol with 10mM ammonium formate used at the flow rate of 60 μL/min. Mass spectrometric detection was performed by an Orbitrap Velos Pro (Thermo Fisher Scientific) at the resolution power of 100,000 with ESI at negative ion mode. Peaks were extracted from LC-MS spectra using MZmine-2.6, and we identified peaks by comparing with database (kegg http : //www.kegg.jp/kegg/, LIPID MAPS http : //www.lipidmaps.org/).

RESULTS AND DISCUSSIONS
First, we compared the extract of cells stimulated for 30 minutes and not stimulated. About 20000 peaks were obtained from the cellular extract, and t-test was performed for these peaks to clarify compounds increased or decreased in stimulated cells. Compounds, for example lipids and saccharoids, differed significantly were identified by database search using exact mass. Peaks such as *m/z* 341.1098 ($C_{12}H_{22}O_{11}$, disaccharide) and *m/z* 508.4735 ($C_{32}H_{63}NO_3$, ceramide) decreased in stimulated cells, and peaks such as *m/z* 238.0946 ($C_9H_{13}N_5O_3$, dihydrobiopterin) and *m/z* 508.4735 ($C_{43}H_{81}O_{13}P$, phosphatidylinositol) increased. Especially we picked up the peak *m/z* 335.2228 ($C_{20}H_{32}O_4$, a compound in arachidonate cascade), it increased in stimulated cells. It is known that lipid mediators such as prostaglandins and leucotrienes are released after degranulation in mast cells. Therefore it seems that this result reflects increase of prostaglandin and leukotriene-synthesis.
Next, in order to investigate time dependent change in detail, we measured the extract of cells stimulated for 5 minutes or 10 minutes. As a result, it was shown that arachidonic acid peak *m/z* 303.2330 increased in 5 minutes samples and 10 minutes. It suggests that arachidonic acid-synthesis increased at 5 minutes, and its synthesis and its consumption in arachidonate cascade are in equilibrium around 5 minutes to 10 minutes. In addition, phospholipid peaks changed such as *m/z* 834.5291 ($C_{46}H_{77}O_{10}NP$, phosphatidylserine, increased) and *m/z* 744.5549 ($C_{41}H_{79}NO_8P$, phosphatidylethanolamine, decreased) after stimulation.
Identification of compounds indicated above by MS/MS and more detailed analysis are in progress.

PTu-182
13:30 – 14:40

Brain proteomics reveals early molecular signature of pathology in pre-symptomatic mouse model of Alzheimer's disease

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Keywords:
shotgun proteomics, mass spectrometry, LC/MS, amyloid-beta peptides

Novel aspects:
These data confirm that AD-typical molecular pathways can be detected already in pre-symptomatic mice expressing one of the major amyloid-beta peptides.

Abstract:
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of extracellular amyloid plaques composed of amyloid-β (Aβ) peptides. Aβ aggregation could be triggered by naturally occurring abnormal forms of Aβ peptide, including N-truncated forms, as well as overproduction of Aβ peptides in brain. Analysis of amyloid deposits in AD brains revealed various N and C terminal variants. Among them, truncated Aβ peptides with pyroglutamate at third amino acid position (Aβp$_{E3-42}$) are gaining considerable attention as potential key players in the pathogenesis of AD. Aβp$_{E3-42}$ is abundant in AD brain and has a high aggregation propensity, stability and cellular toxicity. A novel AD mouse model TBA42 that expresses soluble Aβp$_{E3-42}$ leading to an age-dependent neurological phenotype without aggregating in plaques has been generated. This model is well suited to study the role of soluble Aβp$_{E3-42}$ without possible interference of other toxic Aβ peptides, either full-length or N- and C-variants.
Since early events are critical in the development of AD-related pathology, it is important to understand the molecular mechanism behind these events. Here we studied the early, pre-symptomatic proteome changes in the brain of 4-month old TBA42 female mice compared to the wild-type (WT) controls. We complemented the in-gel tryptic digestion protocol with the filter-aided sample preparation (FASP) method. The proteins found significantly changed in both types of sample preparation represent the most reliable group. To put the results in the context of known signaling pathways, we performed quantitative pathway analysis using the pathway search engine approach developed by us earlier.
Label-free proteomics was used to identify and quantify 858 proteins using in-gel digestion and 521 proteins using FASP digestion. Several proteins were found to be significantly regulated in both in-gel and in-solution analysis including Homer proteins involved in scaffolding, organizing proteins at the synapse and regulating intracellular calcium within the neuron. Moreover, analyzing key nodes, which are regulatory molecules found on pathway intersections, we identified Rho-kinase (ROCK), a serine/threonine kinase and one of the major downstream effectors of GTPase Rho, as well as three key nodes of the mTOR/p70S6K signaling pathway previously implicated in multiple fundamental biological processes including synaptic plasticity and up-regulated in AD.

PTu-184

13:30 – 14:40

Realtime molecular analysis of allergenic response in single mast cell by fluorescence probe-assisted Live Single-cell MS

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Keywords:
Single cell, nanospray, RBL-2H3, Fluorescent probe, Direct analysis

Novel aspects:
Specific peaks of allergy reaction-linked molecular were clarified by direct and realtime analyses in a live single-cell.

Abstract:
< Introduction >
Biological studies usually use plenty amount of samples although cell populations are almost heterogeneous and asynchronous even after they are added with some stimuli. When extracellular stimuli are added on cell surface such as antigen binding to its receptor, sequential response occurs in/around cellular membrane assumed by certain proteins and small molecules to induce secondary response inside the cytoplasm such as calcium efflux from endoplasmic reticulum. These responses sometimes terminate in a short time, and begin and last differently among cells. Thus, analyses using large numbers of cells in these sharp signaling responses sometimes give ambiguous results, whereas single cell analyses prove this problem. Live Single-cell MS allows realtime recovery and direct mass spectrometric analysis of a targeted cell under a microscope using a nanospray tip. We applied this method to follow signal transduction monitored by fluorescent probe for second messenger.

< Methods >
We used rat basophilic leukemia cell line, RBL-2H3, to monitor stimuli-induced molecular change. First, RBL-2H3 cells were inoculated onto feeder cells (fibroblasts ceased proliferation by cytotoxic agent) to induce cellular maturation. Before inoculation, cells were stained with a lipophilic fluorescent probe to distinguish from feeder cells. After inoculation, maturated cells were pre-stained with fluorescent indicator such as Fluo 4 to monitor secondary intracellular response. Cells were inoculated with anti-dinitrophenol (DNP) antibody to produce antigen receptor. Then, the cells were stimulated with DNP to start the antigen siglal. In some case, cells were stimulated with 1 mM of calcium ionophore A23187 or 100 ng/mL 12-O-Tetradecanoylphorbol 13-acetate (TPA). Stimulated cells were observed under a fluorescent microscope (IX-70, OLYMPUS) equipped with excitation and emmition filters for green (Fluo 4) and orange fluorescence (lipophilic RBL-2H3 indicator). Fluo 4 fluorescence was continuously observed and a fluorescence-increased (stimulus-responded) cell was sucked with a nanospray tip of 3 μm bore (Cellomix tip, HUMANIX) followed by addition of ionizing solvent from the backend and analyzed by a LTQ Orbitrap XL (Thermo Fischer Scientific). Peaks obtained with full scan or selected ion monitoring (SIM) mode were extracted and evaluated.

< Results and Discussion >
We continuously observed Fluo 4 fluorescence under a fluorescent microscope after stimuli and the fluorescence increased in some cells. The fluorescence-increased cell was sucked with a nanospray tip. Then, ionizing solvent (1% NH3, 9% water, 90% acetonitrile) was added from the back-end of the tip and the tip was enforced to nano-ESI analysis at negative ion mode of -800V spray voltage. The result showed that peaks derived from intracellular Fluo 4 such as *m/z* 1023.2488 ($C_{48}H_{45}F_2N_2O_{21}$), *m/z* 807.1854 ($C_{36}H_{29}F_2N_2O_{13}$) were detected. We used these peaks as a maker to confirm that content in a cell were micro-sucked successfully, because accumulation of Fluo 4 was observed under a fluorescence microscope. In addition, peaks such as *m/z* 508.4765 ($C_{32}H_{63}NO_3$, Cermides) and *m/z* 835.5342 ($C_{43}H_{81}O_{13}P$, Phosphatidylinositol) were also detected. The former decreased, and the latter increased after stimuli. These results provide that the change of intracellular molecules by extracellular stimuli could be analyzed in realtime in responded cell by monitoring with fluorescent indicators in intracellular signal transduction of allergic reaction in a live single RBL-2H3 cell. For detection of specific peaks after stimulation, the negative ion analysis in a single stimulated RBL-2H3 cell was now in progress.

PTu-185
11:10 – 12:20

A host-pathogen interaction network study of Shigella flexneri infection of human cells targeting key metabolic enzymes

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Keywords:
srm, pathway, host-pathogen interaction network

Novel aspects:
Identification and quantification of key metabolic enzymes in host pathogen interaction

Abstract:
Infectious diseases are a major cause of morbidity and mortality worldwide. Growing resistance to currentant microbials and lean pipelines for novel therapeutics increasingly limit treatment options. However as infectious diseases are usually the consequence of a fight between two networks of hundreds to thousands of individual factors and not just a single mechanism, interfering with any single factor often has insufficient effects on infection outcome and an integrated system-level analysis is thus required for rational development of novel control strategies. This project focuses on metabolism as a subsystem of the HeLa-*Shigella flexneri* interaction network, aiming to quantify as many and specific changes in abundance in the metabolic proteins upon various conditions of infection.
The discovery workflow is adjusted to enable the maximum number of proteins to be identified with a standard LC-MS/MS setup. A quicker approach for obtaining approximate quantities of large subsets of proteins across several cell states has been established. To allow for more accurate quantitative data and absolute quantification, SRM with stable isotope labeling is applied to sets of target enzymes. These techniques are implemented on samples from various stages of infection, i.e. time-course studies, as well as on different mutants of the human HeLA cell line and *Shigella* siRNA knockdown samples.

PTu-186
13:30 – 14:40

A highly specific and sensitive determination of the sterols in silkworm larva by HPLC/APCI-MS/MS for the investigation to insect steroidogenesis.

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Keywords:
HPLC/APCI-MS/MS, Cholesterol, Phytosterol, Silkworm, Ecdysone

Novel aspects:
APCI-MS/MS was able to ionize intact cholesterol and phytosterols, allowing simple and rapid sample preparation and simultaneously quantified the sterols in the silkworm larval tissues in the femtomole range.

Abstract:
The biochemical quantification of sterols in insects has been difficult because only small amounts of tissues can be obtained from insect bodies and because sterol metabolites are structurally related. We have developed a highly specific and sensitive quantitative method for determining of the concentrations of seven sterols- 7-dehydrocholesterol, desmosterol, cholesterol, ergosterol, campesterol, stigmasterol, and β-sitosterol -using cholesterol-3,4-13C_2 as an internal standard. The sterols were extracted from silkworm larval tissues using Bligh and Dyer method and were analyzed using a high performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (HPLC/APCI-MS/MS) . APCI was able to ionize intact sterols at atmospheric pressure, allowing more rapid and simple sample preparation. Tandem mass spectrometry using a triple quadrupole mass spectrometer was performed by selected reaction monitoring (SRM) , detecting m/z 145 or 147 fragment ion which includes the A and B rings of the dehydrated ion of each sterol. The method achieved simultaneous analysis of most of the sterols with femtomole sensitivity.

The newly acquired method can be utilized for the investigation to the molecular mechanism of steroid hormone biosynthesis in insects. In this report, we focused on the cholesterol uptake mechanism in steroidogenesis organ, prothoracic gland. The cholesterol is a starting material for the steroid hormone, ecdysone which regulates the timing of insect molting. However, the molecular identity of the cholesterol supply mechanism has remained elusive. Because insects cannot synthesize sterols *de novo* from acetate, each type of insect could have developed abilities to absorb sterols at the cellular levels to maintain optimum ecdysteroid biosynthesis. We first analyzed the sterol concentration changes in prothoracic glands based on presence or absence of ecdysone secretion. The data revealed free cholesterol and dietary phytosterols accumulation in the ecdysone biosynthesis stages. Second, we analyzed the function of LpR, one of the transporters expressed in the prothoracic glands, and revealed that LpR could uptake the sterols from the plasma lipoprotein. In the future work, we will try to analyze the transporter function more in detailed and search its regulator with utilizing quantitative performance of the HPLC/APCI-MS/MS system, elucidating the nature of the cholesterol uptake mechanism in the insect steroidogenesis organ.

A simple, sensitive and specific method was successfully developed for the quantification of the sterols concentrations in each tissue of an individual silkworm larva. The method was also a useful tool for the investigation of the cholesterol transporter function. This method will be a useful for further investigation to molecular basis of sterol physiology in insects, facilitating the quantification of femtomole quantities of sterols in biological samples.

PTu-187 Evolved gas analysis of a Japanese lacquer film

11:10 – 12:20

Noriyasu Niimura

JEOL, Tokyo, Japan

Keywords:
Japanese lacquer, urushiol, field ionization

Novel aspects:
It is the first time that field ionization mass spectrometry has been applied to the evolved gas analysis of a Japanese lacquer film for the structural study.

Abstract:
Japanese lacquer is sap of lacquer trees. The sap is latex composed of urushiol, water, plant gum, glycoprotein and laccase enzyme. It dries into a tough film, so it is used as a coating material for wood and pottery. The lacquer film, which is urushiol polymer, is insoluble. Therefore, analytical methods are limited. The evolved gas analysis is one of the methods. Field ionization (FI) mass spectrometry is an effective method for evolved gas analysis, because it preferentially produces molecular ions and, in some cases, protonated molecular ions. There is less fragmentation in FI than in electron ionization (EI) , therefore, FI mass spectrum of evolved gas is simpler than EI mass spectrum.

Using EI and FI mass spectrometry, the evolved gas from a Japanese lacquer film at 320 &#8451 ; was analyzed and two results were compared. We previously reported that the thermogravimetry (TG) curve of the lacquer film shows the weight decrease in two stages, i.e., the thermal degradation gradually begins at around 200 &#8451 ; , and the degradation rate becomes fastest at 400-500 &#8451 ; . The derivative thermogravimetry (DTG) curve shows two peaks at 320 &#8451 ; and 470 &#8451 ; . The evolved gas at 320 &#8451 ; contains the thermal degradation product from the surface of the lacquer film.

The EI mass spectrum was complicated because it was consisted of peaks due to the various kinds of fragment ions as well as molecular ions of the thermal degradation products. We compared the observed spectrum to that of the evolved gas from 3-pentadecylcatechol, which is a component of urushiol. The molecular ion was detected at *m/z* 320, and the typical fragment ion was detected at *m/z* 123 as the base peak in the mass spectrum. The molecular ion gives rise to the fragment ion at *m/z* 123 by preferential cleavage at β to the aromatic ring. Both the molecular ion and the fragment ion were also detected in the mass spectrum of the lacquer film. These ions are attributed to 3-pentadecylcatechol, which is a thermal degradation product of the lacquer film. The lacquer was reported to be hardened by laccase-catalyzed oxidative coupling of urushiol and autoxidative cross-linkage of the side chains. In these reactions, saturated side-chains serve as terminal groups of the polymer, whereas active unsaturated side-chains like alkenylcatechol form polymer skeletons. Therefore, 3-pentadecylcatechol is preferentially dissociated out by the thermal degradation from the terminal groups at the lower temperature around 320 &#8451 ; .

In the FI mass spectrum of the evolved gas from the lacquer film, the molecular ion of 3-pentadecylcatechol was also detected as the base peak. This result indicates that the gas consists of 3-pentadecylcatechol, which supports the result obtained using the EI mass spectrometry. Several peaks were also detected at the lower *m/z*. These peaks could not be attributed to 3-pentadecylcatechol, because any of these peaks were not detected as fragment peaks in the FI mass spectrum of the evolved gas from 3-pentadecylcatechol. Two of these peaks were identified as the molecular ion of hydroxymethylfurfuraldehyde and protonated molecular ion of anhydrosugar. The lacquer film is composed of plant gum. The constituent of the plant gum is polysaccharide and its principal constituent sugar is galactose. It has been reported that galactose is thermally degraded to hydroxymethylfurfuraldehyde and anhydrosugar. Therefore, the observed hydroxymethylfurfuraldehyde and anhydrosugar were reasonably identified as thermal degradation products of plant gum. It is concluded that the plant gum is concentrated in the surface of the lacquer film and it is thermally degraded at the lower temperature around 320 &#8451 ; .

PTu-188
13:30 – 14:40

Structural characterization of ethylene-vinyl acetate copolymers using high-resolution MALDI-TOFMS with a spiral ion trajectory

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Keywords:
EVA, Spiral-TOFMS, polymer characterization

Novel aspects:
Detailed structural characterization of ethylene-vinyl acetate copolymer (EVA) could be performed by MALDI Spiral-TOFMS. The high-resolution mass spectra enabled us to evaluate the profiles of thermo-oxidative degradation of EVA.

Abstract:
Ethylene-vinyl acetate copolymer (EVA) is a typical polymer encapsulant of a solar photovoltaic (PV) module. Because the EVA encapsulant serves as a sealant protecting silicon solar cell from moisture, degradation of EVA strongly affects the lifetime of PV modules. In general, degradation of EVA is initiated by deacetylation at vinyl acetate (VA) units, forming polyunsaturated hydrocarbon chains. Under an oxidative condition, the formation of ketones is also possible. Although degradation profiles and mechanisms of EVA have mainly been investigated by means of thermal analysis and Fourier transform infra-red absorption spectroscopy (FT-IR) , these techniques give less information on the chemical structure of each polymer chain.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is commonly used as a technique for polymer characterization. However, the structural characterization of EVA is a challenging task by using a conventional TOFMS, since the random nature of comonomer distribution generates very complicated mass spectra, in which the peaks of different chemical compositions with the same nominal mass overlap. In this study, a detailed structural characterization of EVA was attempted using a high-resolution TOFMS instrument with a spiral ion trajectory (Spiral-TOFMS) .

The EVA samples with different VA composition (12-40 wt%) were used. Because each EVA sample has wide molecular weight distribution, fractionation by size-exclusion chromatography (SEC) was performed. The fractions of the oligomer region (> ca. 3000 Da) were subjected to MALDI measurements. DCTB matrix and NaI cationization salt were used to generate $[M + Na]^+$ ions. MALDI mass spectra were observed using a Spiral-TOFMS (JEOL) .

Complicated mass spectra were observed due to their random comonomer distribution. The monoisotope peaks of EVA chains having ethylene (E) with m units and VA with n units (E_nVA_m) were overlapped with the isotopes of ($E_{n+3}VA_{m-1}$) , whose mass differences were only 0.06 Da. The peak resolution of 40,000 - 60,000 (FWHM) could be achieved by MALDI Spiral-TOFMS, enabling the separation of a number of isobaric peaks with different copolymer compositions and the assignment of each peak to a unique component.

The mass spectra of the degraded EVA samples were much more complicated, because the isobaric peaks of deacetylated and/or oxidized products with different comonomer compositions overlapped with those of intact components with ca. 0.07 Da diffrence. Even for the complicated mass spectra, detailed chemical structures (comonomer compositions and structural changes) of each polymer chain could be characterized. The compositional distribution of EVA before and after degradation were evaluated using a Kendrick mass defect (KMD) plot, which can depict the distribution of mass deficiency vs mass. The degradation of EVA chains will form unsaturated bonds and/or ketone groups, resulting that mass deficiency from the mass of saturated hydrocarbon chains increase. Therefore, the KMD plot could reveal the degradation profiles of EVA. In conclusion, MALDI Spiral-TOFMS has potential as a powerful tool for the structural and compositional characterization of EVA copolymers.

PTu-189

11:10 – 12:20

Determination of Volatile Organic Compounds in Polypropylene Raw Materials by Thermal Desorption-Gas Chromatography/Mass Spectrometry and Head Space-Gas Chromatography-Mass/Spectrometry

Shuqi Sun, Ying Zhang, Song Chen, Yang Song

SINOPEC Beijing Research Institute of Chemical Industry, Beijing, China

Keywords:
VOCs, HS-GC/MS, TD-GC/MS

Novel aspects:
The TD-GC/MS methods was used in the determination of volatile compounds emitted from polypropylene (PP) raw materials, and campaired with HS-GC/MS method.

Abstract:
Thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) and head space-gas chromatography-mass/spectrometry (HS-GC/MS) had been applied to the determination of volatile compounds emitted from polypropylene (PP) raw materials.

During the TD analysis, 0.30g of sample was heated in a stainless steel tube at 90&#8451 ; for 3 min, the emitted volatile substances was cooled with the help of a helium gas stream in the cold trap. After completion of the baking phase, those absorbed in the cold trap are rapidly heated from -10&#8451 ; to 280&#8451 ; . The target compounds were evaporated and then isolated in the gas chromatographic separating column and detected by the mass spectrometer. Chromatographic separation was carried out on an HP-5MS column (30m×0.25μm×0.25mm) with a temperature program, and the mass spectrometric detection was operated by a quadrupole mass spectrometer with an electron impact ion source. The mass spectrums of each chromatograph peak obtained from the TD-GC/MS method were searched in the NIST Mass Spectral Library to obtain the qualitative results.

And the HS technique had been carried out at 120&#8451 ; for 5 hours, 4.50g PP raw materials were sealed in a 20mL HS glass bottle which was heated in the head space sampler. The head space gas which composed of air and the volatile matter were injected by the headspace sampler into the inlet of the gas chromatography. The GC/MS experimental conditions were the same as described above.

A series of PP raw materials were detected by both of HS-GC/MS and TD-GC/MS analysis methods. The research results showed that the volatile organic compounds (VOCs) released from the PP raw materials were composed of alkanes, alkenes and oxygen compounds with low molecular mass. Compared with HS method, the results of TD technology attained a large number of chromatographic peaks from which indicated that more substances would be determinate and showing a wide application prospect in the determination of the VOCs of the PP raw materials.

PTu-190
13:30 – 14:40

Characterization of a multi-component liquid crystalline copolyester by high-resolution MALDI-spiral-TOF-MS

Banri Hashimoto, Takanori Sasaki, Hajime Ohtani

Nagoya Institute of Technology,Aichi,Japan

Keywords:
charactrization, MALDI-spiral-TOF-MS, LCP, copolymer

Novel aspects:
high resolution MS study to characterize copolymer systems

Abstract:

[INTRODUCTION]

In recent years matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) has been widely utilized in the field of characterization of synthetic polymers. In the case of copolymer samples, however, their structural characterization is generally difficult by ordinary MALDI-TOF-MS mainly because of its insufficient resolution for complex composition and chemical structures of the copolymers. In this work, by using recently developed high-resolution MALDI-spiral-TOF-MS, a liquid crystalline terpolyester (LCP) composed of p-hydroxybenzonic acid (HBA) , isophthalic acid (IA) , and p-aminophenol (PAP) was characterized in detail.

[EXPERIMENTAL]

The LCP sample was dissolved in hexafluoro-2-propanol (5 mg/mL) to prepare the sample solution. An aliquot (1 mL) of the sample solution was mixed with 10 mL of the matrix solution (1,8,9-trihydroxyanthracene 20 mg/mL in THF) . 1 mL solution of cationizing reagent (NaI, 10 mg/mL in THF) , and then 1 mL of the sample/matrix mixture solutions were stepwise spotted on the sample plate. High-resolution MALDI mass spectra of the LCP sample were acquired in spiral mode using a JEOL/JMS-S3000 time of flight mass spectrometer.

[RESULTS AND DISCUSSION]

In the MALDI mass spectrum of the LCP sample, several series of peaks were observed with the interval of m/z 120 which corresponds to the molar mass of a HBA unit. Moreover, in an expanded mass spectrum, isotopic peaks of a component in the LCP sample were clearly separated. For example, a group of isotopic peaks was observed from m/z 1040 to 1044. Since measured accurate mass of m/z 1040 peak is 1040.2220, this component was identified to be a monoisotopic ion of HOOC-C_6H_4O-(IA)$_3$-(PAP)$_3$-(HBA)-$COCH_3$ (calculated exact mass, m/z 1040.2273) . Meanwhile, the relative intensities of the isotopic peaks (m/z 1041-1044) to the monoisotopic ion peak (m/z 1040) observed in the MALDI mss spectrum was significantly larger than the calculated intensities based on the theoretical isotopic pattern. This fact suggests that the peaks larger than m/z 1041 were not only composed of the isotopic ions for m/z 1040 monoisotopic ion but also overlapped with other monoisotopic ions. From accurate molar mass of each peak, corresponding monoisotopic ions were then identified to be a HOOC-C_6H_4O-(IA)$_2$-(PAP)$_2$-(HBA)$_3$-$COCH_3$ (m/z 1041) , and HOOC-C_6H_4O-(IA)-(PAP)-(HBA)$_5$-$COCH_3$ (m/z 1042) . Moreover, by comparison between measured and theoretical intensities for the corresponding isotope clusters, relative abundance of each monoisotopic ion in the observed peak was estimated to be 92.9% in m/z 1040, 17.0% in m/z 1041, and 19.7% in m/z 1042 respectively. Basically in a similar manner, various peaks observed in the MALDI mass spectrum were successfully interpreted in terms of peak identification and relative abundances of the LCP components.

PTu-191 Mass-analysis of enzymatic hydrolysis of nylon by argon cluster SIMS

11:10 – 12:20

Kensuke Iuchi, Kosuke Moritani, Kozo Mochiji, Keisuke Nagai, Kazuki Iida, Seiji Negoro

University of Hyogo, Hyogo, Japan

Keywords:
Ar cluster SIMS, cluster ion, enzymatic hydrolysis, nylon

Novel aspects:
This is the first attempt to apply SIMS to evaluation of hydrolysis of nylon.

Abstract:
In the secondary ion mass spectrometry (SIMS) of organic substances, the molecular weight of the intact ions currently detectable is at best only as high as 1000, which for all practical purposes prevents the technique from being applied to organic materials of higher mass. We have developed SIMS equipment in which the primary ions were argon (Ar) cluster ions. Ar cluster ions consist of hundreds to thousands of Ar atoms aggregated by Van der Waals forces. The average kinetic energy per atom in the cluster ion is equal to the energy used to accelerate the cluster ion divided by the number of atoms in the cluster ion. For example, when a cluster of 1000 atoms is accelerated to 10 keV, the average kinetic energy per atom of the cluster ion is only 10 eV. Thus a large cluster ion can generate many low-energy constituent atoms in a collision with the surface. The intensity of fragment ions of several organic molecules was decreased by a factor of 10^2 when the kinetic energy per atom was decreased below 5 eV. Moreover, intact ions of insulin (molecular weight : 5,808) and cytochrome C (molecular weight : 12,327) were successfully detected without using any matrix [1]. These results indicate that fragmentation can be substantially suppressed without sacrificing the sputter yield of intact ions when the kinetic energy per atom is decreased to the level of the target's dissociation energy.

We applied the Ar cluster SIMS to mass-analysis of the enzymatic hydrolysis of nylon. Nylon are synthetic polymers that contain recurring amide groups (R-CO-NH-R') as integral parts of their main polymer chains. The high strength, elasticity, abrasion resistance, chemical resistance, and shape-holding characteristics of nylons over wide temperature ranges make these polymers suitable for the production of fibers and plastics. On the other hand, methods to recycle nylons or decrease the waste of them at low cost are required. Biodegradation of nylon is a strong candidate from environmental view point. We have investigated the enzymatic hydrolysis of nylon [2]. The enzyme studied here is 6-aminohexanoate oligomer hydrolase (NylC) which is extracted from bacteria. This enzyme is a member of the N-terminal nucleophile hydrolase superfamily that is responsible for the degradation of the nylon-6 industry byproduct. We prepared thin film of nylon oligomer, ACO (6-aminohexanoate-cyclic-oligomer) on silicon substrate. Enzyme reactions were performed by dipping the nylon samples in the enzyme solution at 60&#8451 ; for 2 h. Secondary ion mass spectra of the sample before and after enzyme reaction were measured. As a result, the intensity of several components of the nylon oligomer was significantly decreased by the enzyme reaction. The experiments using more highly polymerized nylon are in progress. We propose that NylC should be designated as a nylon hydrolase and Ar cluster SIMS is promising tool to evaluate the enzymatic degradation of nylon.

[1] K. Mochiji, et al., *Rapid Commun. Mass Spectrom.* **23**, 648 (2009).
[2] S. Negoro, et al., *J. Biol. Chem.*, **287**, 5079 (2012)

PTu-192
13:30 – 14:40

Characterization of poly(ethylene oxide-propylene oxide) alkyl ether (R-EO-PO) copolymer by LC-ESI-MS

Haruka Yamada[1], Hirotaka Hisatomi[1], Mendra Ritonga[2], Sana Ito[2], Masaki Morita[2], Hideya Kawasaki[1], Ryuichi Arakawa[1]

[1]Kansai University, Suita, Japan, [2]Niitaka Co., Ltd., Osaka, Japan

Keywords:
poly (EO-PO) alkyl ether (R-EO-PO) , EO/PO copolymer, nonionic surfactant, structural analysis, LC-ESI-MS

Novel aspects:
The numbers of carbons in the alkyl chain R, EO and PO units for R-EO-PO copolymers were unambiguously determined by LC-ESI-MS.

Abstract:

Introduction

Mass spectrometry (MS) has become increasingly important in the characterization of synthetic polymers. For the MS analysis of the polymers, different ionization methods have been used, especially matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) . The both methods are well suited for the determination of the number of repeat units, end groups, linear or cyclic structure, and molecular weight distribution of homopolymers. However, for the MS analysis of copolymers, it is much more difficult to obtain the structural information, because their mass spectra are extremely complicated. Recently, a few LC/MS applications with ESI or APCI ionization have been reported concerning the characterization of copolymers. It is expected that mass spectra of copolymers are simplified by LC separation. In this study, poly (ethylene oxide-propylene oxide) alkyl ether (R-EO-PO) was characterized in detail using poly (ethylene oxide) alkyl ether (R-EO) as a reference of LC-MS data by LC-ESI-MS.

Methods

The LC-MS data were obtained with an Exactive orbitrap mass spectrometer and Accela LC system (Thermo Fisher Scientific Inc.) with a column of Inertsil ODS-3 3 um 2.1×150mm (GL Science) . The best LC separation for the R-EO and R-EO-PO polymers was achieved using a gradient method with 5 mM ammonium acetate aqueous solution and acetonitrile/THF solution (1/1, v/v) .

Preliminary data

The LC-MS data plot was shown with the column retention times as x-axis, the m/z values as y-axis and the ion intensities adjusted with the color density as z-axis. The data plot for the R-EO-PO copolymer exhibited clear distributions of the peaks with m/z differences of 44 and 58 (singly charged) along the y-axis attributed to the masses of EO (44 Da) and PO (58 Da) . The distributions along the retention time (x-axis) originate from alkyl chains (R) and PO chains with an ODS column ; the longer alkyl chains (R) and the PO chains, the more hydrophobic the copolymer and the longer the retention times become. The hydrophobic property increases in the order of EO<PO<R. In other words, the R-EO-PO copolymer was separated based on the number of carbons in the alkyl chain, EO and PO units. However, the mass difference 14 Da between PO and EO is equal to CH_2 repeating unit in an alkyl chain R, so that the same m/z isomers with different combinations of CH_2 (R) , EO and PO units are considered. These isomers cannot be distinguished from the m/z values. To solve the problem, the R-EO polymer without PO was used as a reference for the LC-MS data plot. The numbers of carbons in R and EO units for the R-EO polymer were unambiguously determined from the m/z values. Therefore, comparing the LC data plot of the reference R-EO with that of R-EO-PO, we could successfully determine the numbers of carbons, EO and PO for the R-EO-PO copolymers using LC-ESI-MS.

PTu-193
11:10 – 12:20

Novel Method for Verification of Trace Amounts of Polymer Contaminants in Photoresist Solution

Min-Soo Suh, Da-Hee Lee, Taehyo Choi, Minjung Kim, Dongju Oh, Jung Dae Park, Pilkwon Jun, Jong Soo Kim

Samsung Electronics, Hwasung, Korea

Keywords:
Polymer impurity analysis, photoresist, Headspace gas chromatography mass spectrometry (HS-GC/MS) , Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Novel aspects:
Rapid and reliable strategy to identify and quantify the polymer impurity in photoresist solution by using two types of mass spectrometry

Abstract:
The levels of organic and inorganic impurities are controlled to minimize the defect and maximize the manufacturing yield in semiconductor business, because even a few ppb-level impurities generate various defects. Hence, an adulteration of polymer solution occurred by human or instrumental errors must be a severe problem. The verification of the origin of a trouble is the key for preventing a recurrence of the mixing problem.
In this study, headspace gas-chromatography mass spectrometry (HS-GC/MS) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS were used to identify the source of polymer impurities. Although GC/MS is not compatible with high-mass organics such as polymers, HS-GC/MS was employed to identify the solvents of polymer without damages on GC column and narrow down the candidates of impurity. Considering the ratio of contents and the molecular weights of polymer and solvents, the number of moles of solvent molecule was much larger than that of polymer, which helped the qualitative and quantitative analysis of trace amounts of contaminants. The solvent contaminant was identified with the scan mode of HS-GC/MS and quantified by monitoring the selected ions (m/z 59) . To convince the identification results at a polymer level, the contaminated polymer solution was measured by MALDI-TOF MS using the optimal analysis condition for the impurity candidate determined by HS-GC/MS. Based on the HS-GC/MS and MALDI-TOF MS results, the route of contamination were determined and blocked, which resulted in disappearance of the bridge-type defects.
The combination of solvent analyzer (HS-GC/MS) and polymer analyzer (MALDI-TOF MS) gives a rapid and reliable solution for the identification and quantification of the ppm-level polymer contaminants.

PTu-194

13:30 – 14:40

Differential Analysis in sulfenamide-based vulcanizing accelerators for rubber products by High mass Accuracy MS and Multivariate Statistical Technique

Takahiro Goda, Hiroki Nakajima, Satoshi Yamaki, Tsutomu Nishine, Masaru Furuta, Naoki Hamada

Shimadzu Co., Kyoto, Japan

Keywords:

vulcanizing accelerator, Differential Analysis, high mass accuracy MS, multivariate statistical technique, searching out structural analogues

Novel aspects:

The structures of impurities which were structural analogues of main compounds in vulcanizing accelerator reagent were identified by high mass accuracy MS and multivariate statistical technique.

Abstract:

Vulcanization is a cross-linking reaction for forming bridges between individual polymer chains via addition of sulfur. The purpose of vulcanization is to convert rubber into more durable materials. In general, a reaction rate of vulcanization is increased by adding vulcanizing accelerator to mixture of rubber and sulfur. Each tire manufacturer employs particular one from among a lot of commercially available reagents by their own evaluation criteria. Therefore, analyzing them is a crucial in the development of tires. However, it is difficult to detect differences in the reagents which have the same main compounds produced by different manufacturers. Here, we show the differential analysis of sulfenamide-based vulcanizing accelerators which have the same main compounds produced by different manufacturers using high mass accuracy MS^n and multivariate statistical technique.

Five N- (tert-butyl) -2-benzothiazole sulfenamide (NS : NS-1, NS-2, NS-3, NS-4, NS-5) and five N-cyclohexyl-2-benzothiazole sulfenamide (CZ : CZ-1, CZ-2, CZ-3, CZ-4, CZ-5) were used in this study. Each NS and each CZ were produced by different manufacturers. Sample solutions were prepared at 100 mg/L with tetrahydrofuran and acetonitrile. Equal amount of NS solutions were mixed and it was used as a quality control (QC) sample for NS analysis to identify the robustness and reproducibility of the analytical data. The QC sample for CZ analysis also was prepared by the same method. LCMS measurement was conducted using LCMS-IT-TOF (high performance liquid chromatograph / ion trap time-of-flight mass spectrometer, Shimadzu Corporation) . SIMCA-P+ software (Umetrics) was used for multivariate statistical analysis. MetID Solution software (Shimadzu Corporation) was used for searching out structural analogues using MS^n data acquired by LCMS-IT-TOF measurement. Formula Predictor software (Shimadzu Corporation) was used for predicting the molecular formulae of the statistical analogues.

As a result of principal component analysis (PCA) for NS, the groups of each sample were located at the different sites on the score plot. And, the unique peaks of each sample were observed on the loading plot. Then, the candidates of structural analogues of NS were searched out from among those unique peaks based on the similarities of fragment ions and neutral losses calculated by partial least square (PLS) . The extracted ion chromatograms (EICs) of the structural analogues suggested that they were characteristic compounds of each sample. The molecular formulae of them were predicted using the accurate mass and isotopic ratio. We also determined the validity of the structures of these compounds using MS^n measurement.

For example, a unique peak of *m/z* 253 in NS-2 was observed on the loading plot. It was cleared to be a structural analogue of NS by the similarities of fragment ions and neutral losses. The molecular formula was predicted as $C_{12}H_{16}N_2S_2$. The same neutral losses as NS were detected, and product ion showing the addition of $-CH_2$ to the phenyl group was observed in MS^4 data of this compound. These results suggested that this compound had the structure of the addition of $-CH_2$ to the phenyl group of NS. The structures of other compounds also were identified by the same procedure. These compounds are considered as the impurity or by-product in the process of synthesis. In the analysis of CZ, we also could determine the structures of impurities or by-products in the process of synthesis by the same procedure.

It was confirmed that the kind of impurities in each sulfonamide-based vulcanizing accelerator differed from manufacture to manufacture.

PTu-195

11:10 – 12:20

Diagnostics of degradation levels for thermoplastics using pyrolysis-GCxGC-TOFMS

Fumie Kabashima, Fumihiko Tsuchiya

LECO Japan Corporation

Keywords:

GCxGC, TOF-MS, Pyrolysis-GC/MS, Highpolymers, Insulating materials

Novel aspects:

TSUGE Shin, Nagoya University OHTANI Hajime, Nagoya Institute of Technology WATANABE Chuichi, Frontier Laboratories Ltd. ,Pyrolysis-GC/MS Data Book of Synthetic Polymers

Abstract:

Abstract

At present, cable ties are used to bundle wires and cables, such as those used in electric devices. When these cable ties are used outdoors, the cable ties gradually become degraded due to exposure to ultraviolet rays, for example. In order to evaluate the degradation level of insulating materials, there are physical tests, such as the loop tensile test. However, there are several problems with this method ; significant variance of the test results, the need to use a lot of samples, and the test itself is a cumbersome and time-consuming task. Therefore we employed pyrolysis-GCxGC-TOFMS as a new method for easily and accurately assessing degradation levels with a small sample. This system separates and identifies the components in the sample according to the separation characteristics of the two columns enables the separation of the components, which could not be separated by using a normal GC system, on the 2nd dimensional chromatograms.

Method and experimental

Three types of cable ties made of polyamide resin were used as test samples ; 2 kind of PA66 and PA11. The artificially degraded samples were made by irradiating ultraviolet rays onto each sample. For the weather resistance test, the Eye Super UV Tester manufactured by Iwasaki Electric Co., Ltd. was used, and the ultraviolet radiation exposure time was set to 0, 50, and 100 hours respectively. The irradiation time of 100 hours corresponds to the outdoor exposure time in one year or more.

Using 2nd dimensional chromatograms of the polyamide resin cable ties, it was confirmed that there were peaks for which the intensities increased as the ultraviolet radiation exposure time increased and peaks for which the intensity was unchanged, without being affected by the degradation. The peak-area ratio was calculated by dividing peak area of the degraded peaks by the standard peaks. A master curve was obtained by plotting the peak-area ratios and ultraviolet radiation exposure time. Separately, the service life was determined by performing a loop tensile strength test on the samples artificially degraded by irradiating ultraviolet radiation. The end of the service life was defined as the point at which the tensile strength has been reduced to the one half of the initial value. By creating a master curve, which shows the relationship between the results of pyrolysis-GCxGC-TOFMS and the loop tensile strength test, it became possible to assess the degradation level of the cable ties made of the polyamide resin samples evaluated in this study. Therefore, using a master curve and calculating the peak-area ratio of the insulating material actually being used, it is expected that the degradation level and the residual life can be accurately determined.

Conclusion

Using this method which enables the correct peak identification, it is possible to quickly evaluate the degradation level with ultralow volume sample. It is expected that this method can also be applied to quality control and the investigation of causes for defective products released to the market. The application of this method for the assessment of the degradation level of insulating materials described in this report is not limited to polyamide resins, but can also be applied to various insulating materials. Moreover, we can be replaced the loop tensile strength test to the flexural strength test or mass-decrease rate.

PTu-196 Yellow Sand and Seawater Interaction from viewpoint of Silica Speciation

13:30 – 14:40

Miho Tanaka[1], Kazuya Takahashi[2]

[1]Tokyo University of Marine Science and Technology, Tokyo, Japan, [2]RIKEN, Wako, Japan

Keywords:
Yellow sand, FAB-MS, silica speciation

Novel aspects:
From our measurement by FAB-MS, it becomes clear that the dimer and linear tetramer are produced at the surface of seawater, when yellow sand falls into seawater, .

Abstract:
1. Yellow sand is a kind of loess and distributed in N. W. China. It is carried eastward by prevailing winds and passes over China, Korea, and Japan. Sulfur, carbon monoxide, and other toxic pollutants including heavy metals (such as mercury, cadmium, chromium, arsenic) often accompany the dust storm, as well as viruses, bacteria, and combustion products. The dust is known to cause a variety of health problems. In Japan, large amounts of yellow sand is mainly transferred in spring. If yellow sand does not contain toxins, it could be a good supplier of silicic acid (silica) to the Sea of Japan, especially in spring. Silica species are nutrients for diatoms in seawater. During spring, silica tends to be lacking due to "blooming " in some regions. The authors have studied dissolution states in aquatic phases, particularly seawater. In this study, the behavior of silica species in yellow sand with model solution of seawater (sodium chloride (NaCl) solution) was examined under several conditions, and the role of yellow sand is considered.
2. Sample of yellow sand was obtained at Hotan in N. W. China by 200 mesh sieve. A portion of yellow sand (0.2g) was shaken with 20 mL of several kinds of sodium chloride at 25°C for several days. The silica concentration was determined using molybdic yellow methods of spectrophotometrically. The chemical species of silica was identified by FAB-MS spectrometry (JEOL JMS 700) (negative ion detection mode) . The conditions for mass spectrometry were as follows : for FAB (Xe, 1 mA emission) , the resolving power was m/m=1000 and mass range was 0 -1000.
3. **pH dependence**The pH of solution was changed from 0.33 to 14.1 and shaken for 7 days. A change in pH from 0.52 to 14.2 of solution containing yellow sand with a 0.5 mol L^{-1} sodium chloride solution was attained. However, the pH before shaking was between 3.2 and 9.0 and remained within 8.1-8.2 after shaking for 7 days. The silica concentration in NaCl was almost a constant 30 μmol L^{-1}. When the pH of the solution was 0.5, 12.4, and 14.2, silica concentration was 1320, 158, and 81 μmol L^{-1}, respectively. When the yellow sand was poured into a NaCl solution, pH equilibrium was attained and NaCl solutions with various pH, were almost constant. In this pH range, the silica species in solution were observed as [monomer-Na^+ complex]$^-$, [monomer-Ca^+ complex]$^-$, dimer, cyclic tetramer and linear tetramer. When pH of the solution was 0.5, peak intensity of silica species was extremely low and the same species were observed. When pH solutions were 12.4, and 14.2, the silica species were clearly observed. The silica concentration in solution with pH 0.5 was high, but silica species not clearly observed. It is considered that silica could be dissolved as particles in the low pH solution but it could be dissolved as ions in high pH.
Time dependence When yellow sand was dissolved for 0, 1, 3 7, and 14 days, the silica concentration increased. For 1 day, dimer and linear tetramer were observed compared to cyclic tetramer. But for 3 days and more, dimer and cyclic tetramer increased compared to linear tetramer. When the solution did not attain equilibrium for 1 day, yellow sand dissolved as linear tetramer first, then changed to cyclic tetramer. According to our previous studies, it has become clear that "food " of diatoms was dimer and linear tetramer. When yellow sand falls into seawater, the dimer and linear tetramer would be produced immediately, at the surface of seawater.

Poster Session

Tuesday, 18th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PTu-197 High Plasmalogen Content in the Gut of Silkworm (Bombyx mori)

11:10 – 12:20

Takako Aboshi, Ritsuo Nishida, Naoki Mori
Kyoto University

Keywords:
plasmalogen, LCMS-IT-TOF, phospholipid

Novel aspects:
We found the high plasmalogen content in the gut of silkworm.

Abstract:
Phospholipids are a major component of membrane and exhibit great diversity in the structure of the apolar and the polar moieties of the lipid molecules. They are distributed unevenly in the tissues and display characteristic phospholipid compositions. Phospholipids perform various functions in the cells and tissues of the body such as natural surfactants, signaling molecules, and antioxidants. The different pattern can be regarded as an adaptation to environment. In this study, we analyzed phospholipid composition in tissues of silkworm, *Bombyx mori* by TLC and LMS-IT-TOF. As a result, we found the ratio of plasmalogen phosphatidylethanolamines to phospholipids was higher in the gut compared to that in other tissues. Plasmalogens are unique subclass of glycerophospholipids characterized by the presence of a vinyl ether at the *sn*-1 position of the glycerol backbone, whereas usual glycerophospholipids have an ester bond at the same position. Plasmalogens are reported to inhibit the metal ion induced oxidation. Midgut tissues suffer from oxidative stress associated with phenolic oxidation in midgut lumen when caterpillars ingest phenolics, known as defense chemicals in plants. Plasmalogens may protect gut tissues against the oxidative stress.

PTu-198

13:30 – 14:40

Characterization on Oxidation and Hydrolysis of Titanium Ion Using ESI-MS

Io Ryumae, Miho Tanaka

Tokyo University of Marine Science and Technology

Keywords:
ESI-MS, titanium identification, dissolution species

Novel aspects:
As identification of dissolution species, oxidation and hydrolysis process of intermediate products of Ti was succeeded by ESI-MS.

Abstract:
Introduction
ESI-MS (Electrospray ionization mass spectrometry) exhibits a soft ionization technique and can deliver ions in solution to gas-phase. Therefore ESI-MS gives information on the dissolution state of metals at molecular level. It is possible to observe chemical change, even if change occurs instantaneously. On the other hand, titanium (Ti) ions in solution exists as Ti (III) and Ti (IV) . Ti (III) is stable in chloride ion (Cl^-) solutions of pH 1 - 2 and Ti (IV) causes precipitation gradually when it is exposed to oxygen in air. For this reason, ESI-MS is an effective technique to measure elements which show similar reactions as Ti ion. Previous studies with ESR and potentiometric titration, the electronic state and dissolution states based on theoretical calculations of Ti ion were observed.
In this study, identification of dissolution species, oxidation and hydrolysis process of Ti with ESI-MS is carried out.

Experimental
ESI-MS (Shimadzu LCMS-2010A) was employed for measurements. The spray voltage was set at +4.5kV or -3.5kV. Ultrapure water (18.2MΩ) was used as the mobile phase. Titanium tri-chloride ($TiCl_3$) , titanium sulfate ($Ti(SO_4)_2$) used were of chemical grade from Wako pure chemical industries, Ltd. Hydrogen peroxide (H_2O_2) used was ultrapure grade from Kanto chemical co Ltd.
To aliquots of 0.1, 1, 3, 5, 10 and 15 mL of $TiCl_3$, were added 50 mL of H_2O_2 and $Ti(SO_4)_2$ solutions. Oxidation and hydrolysis process of Ti (III) using 5 mL $TiCl_3$ by ESI-MS was observed.

Results and Discussion
[1] Speciation of titanium (III) and titanium (IV)
In chloride (Cl^-) and sulfate ion (SO_4^{2-}) solutions (higher than 1 mM) , the observed mass spectra were examined for the singly charged hydrolyzed species of Ti (III) and Ti (IV) in positive ion mode.
For Ti (III) solution, the peaks at *m/z* 154 and 172 proved the existence of $[TiCl(OH)(H_2O)_n]^+$. As there have been no reports of titanium species with Cl^- ligand in positive ion mode, it is necessary to discuss it. Moreover, the existence of the dimer identified as a mixed-valancy species of Ti (III) and Ti (IV) is already reported by ESR. ESI-MS gives information on the intermediate products during the oxidation of Ti (III) to Ti (IV) .
For Ti (IV) solution, previous studies using potentiometric titration had carried out for doubly charged species like TiO^{2+} and $Ti(OH)_2^{2+}$. However, they were not observed by ESI-MS. This indicates that electrochemical reduction occurred due to high voltage in ESI nebulization, which may have changed the dissolution state of titanium species.
[2] Behavior of oxidation and hydrolysis in Ti (III)
For $TiCl_3$ solution, peaks of Ti (III) and Ti (IV) were observed and their peak intensities decreased with increasing time. The peak intensity of Ti (III) decreased more rapidly than that of Ti (IV) . Furthermore, it was observed that the time-dependent peak intensities of Ti (III) / Ti (IV) dropped. Therefore, it was considered that Ti (III) was oxidized to Ti (IV) in solution and Ti (IV) which was hydrolyzed and not present in its ionic form, was rapidly excluded from the solution.

The behavior of oxidation and hydrolysis of Ti (III) could be examined by ESI-MS. On the other hand, there is a possibility of the dissolution state changing during measurements when using ESI-MS. In this presentation, we expect to examine the complete conversion of Ti (III) to Ti (IV) in solution.

PTu-199
11:10 – 12:20

The Development of Multi Elemental Analysis of Ferromanganese Nodule by LA-ICP-MS

Junichi Hirata, Miho Tanaka

Tokyo University of Marine Science and Technology, Tokyo, Japan

Keywords:
LA-ICP-MS, Ferromanganese nodule, Standards, Multi elemental analysis

Novel aspects:
Multi elements in ferromanganese nodules were determined by LA-ICP-MS and the data were compared with those of ICP-MS in order to obtain accurate determination values by LA-ICP-MS.

Abstract:

Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a useful method for determination of trace elements distributions. It shows higher sensitivity than EPMA or SEM-EDX which are also employed for similar purposes. Furthermore, it is possible to observe samples under atmospheric pressure by LA, thereby protecting samples against structural changes. However, the drawbacks of LA-ICP-MS are obtaining. Matched standards as established standards do not exist. Secondly, it is difficult to obtain stable signals and accurate determination values by LA-ICP-MS compared with ICP-MS. The data obtained is more accurate when subjected to internal standard correction and selection of internal standard for the element of interest is to be carefully considered paying attention to the features of the sample. Therefore, the purpose of this study is to obtain accurate determination values by LA-ICP-MS.

Multi elements in ferromanganese nodules, which are one of the notable and important geological samples, were determined by LA-ICP-MS and the data were compared with those of ICP-MS in this work. Certified reference material (CRM) of ferromanganese nodule and manganese dioxide (MnO_2) were used to prepare matrix matched standards. Magnesium which is one of the major elements in ferromanganese nodules was selected for internal standard correction.

Sample preparation

JMn 1 which is a CRM of ferromanganese nodule collected in the Pacific Ocean (purchased from AIST) and high purity MnO_2 powder (99.5 %, Wako Chemical Co.) were used for preparation of standards for LA-ICP-MS. JMn 1 and MnO_2 with the weight ratios of 0, 25, 50, 75, 100% were mixed. These samples were pressed into ϕ 5 mm pellets for IR analysis. Ferromanganese nodule collected in Pacific Ocean near Hawaii (10° 10'N, 147° 00'W, JOGMEC) was cut across the center, and the obtained powder was collected and made into pellets using the same procedure as standards. These pellets and powder are mentioned as Hawaii samples in this paper.

Instrumentation and parameters

UP213 (NEW WAVE) , LA instrument and ELEMENT XR (Thermo Fisher) ICP-MS instruments were used. The conditions of LA are as follows : 213 nm wavelength, 100 µm diameter, 10 Hz repetition rate, 15 µm/sec scan speed, 0.023 mJ pulse energy, and raster sample scan mode. The conditions for ICP-MS were 1.23 L/min sample gas and middle resolution. The elements measured were ^{11}B, ^{24}Mg, ^{52}Cr, ^{56}Fe, ^{60}Ni, ^{63}Cu, ^{95}Mo, ^{111}Cd, ^{140}Ce, ^{153}Eu, ^{175}Lu, ^{208}Pb, and ^{238}U.

Results and discussion

Calibration curves for LA-ICP-MS measurements were successfully obtained. The square of correlation coefficient values (R^2) of the calibration curves were 0.932~0.984. R^2 values of the calibration curves obtained by internal standard correction using ^{24}Mg, which contained 4 points except for blank, were 0.924~0.999. Good linear calibration curves were obtained with internal standard corrections. For example, R^2 value of ^{56}Fe without correction was 0.978, and correction with ^{24}Mg was 0.991. Calibration curves of ^{52}Cr and ^{111}Cd were not obtained due to its low ablation efficiency due to their chemical property or low isotopic abundances.

Accuracy is defined as $[C_{LA\text{-}ICP\text{-}MS}] / [C_{ICP\text{-}MS}] \times 100$ in this study. $[C_{LA\text{-}ICP\text{-}MS}]$ and $[C_{ICP\text{-}MS}]$ are the determination values of the Hawaii sample obtained by LA-ICP-MS and ICP-MS respectively. The accuracy obtained without internal standard correction were in the range 97.4~173%. That with internal standard correction using ^{24}Mg were 80.4~110%. Internal standard correction showed accurate determination values. For example, the accuracy of ^{56}Fe without correction was 135 % and with internal standard correction using ^{24}Mg was 110%. Thus, accurate determination values were obtained by this method.

PTu-200
13:30 – 14:40

Study on Reaction Process of the Complexation of Silicomolybdic and Phosphomolybdic Acids by ESI-MS

Mariko Takahashi, Miho Tanaka

Tokyo University of Marine Science and Technology, Tokyo, Japan

Keywords:
ESI-MS, Silicomolybdic and phosphomolybdic acids

Novel aspects:
ESI-MS as a useful tool to observe complexation reaction processes of silicomolybdic and phosphomolybdic acids.

Abstract:

Introduction

In order to get information on the reaction process of silicomolybdic and phosphomolybdic acids, ESI-MS exhibits a powerful tool. X-ray analysis, NMR and other instruments portray information on these acids, but ESI-MS is able to track each reaction step on addition of reagent. ESI-MS has a soft ionization system and produces multiple-charged ions. Furthermore, any compound may be observed at the same time, when compounds are ionized in ion source. ESI-MS enables us to elucidate reaction process of complexation of silicomolybdic and phosphomolybdic acids in solution.

Silicomolybdic and phosphomolybdic acids are heteropolymolybdic acids, which are used for the determination of silicic acid and phosphoric acid by absorption spectrophotometry known as molybdate yellow and blue methods. These methods were generally used for. These acids are composed of one molecule of silicic or phosphoric acid which is surrounded with 12 molecules of molybdic acids at low pH values. The complexation reaction process of silicomolybdic and phosphomolybdic acids is complicated and has not been elucidated. From previous studies, it has been known that the reaction is affected by the reagent concentration, solution pH, and elapsed time for formation. Therefore, it is necessary to measure not only metal ions but sulfuric acid ion or other ions that affect the complexation in order to examine the complexation reaction process. In this study, we report ESI-MS as a useful tool to observe complexation reaction processes of silicomolybdic and phosphomolybdic acids.

Experiment

ESI-MS (LCMS-2010A : Shimadzu Ltd.) was used in negative ion mode. The mobile phase was ultra pure water.

Generally, the standard solution for silicic acid is prepared using sodium chloride or sodium hydroxide solution with silicon dioxide. In measurements with ESI-MS, the standard solution for silicic acid causes a serious problem. When sample solution contains high concentration of volatile ions with objective ions, the ion suppression of the objective ions occur. To resolve this problem, a new method to dissolve silicic acid in pure water was developed. We use reagent for silicic acid which were obtained by rice nutrient.

Results and Discussion

Silicic or phosphoric acid solution form colored complexes by addition of sulfuric acid and ammonium molybdate solutions. These complexes were measured by ESI-MS. In the reacted solution derived from silicic acid, the peaks assigned as silicomolybdic acid were observed, in addition to molybdic acid. Furthermore, peaks of silicic acid were not observed. Therefore, it indicates that all of silicic acid in solution is converted to silicomolybdic acid. In the reacted solution derived from phosphoric acid, the peaks assigned as phosphomolybdic acid were observed, while main peaks were original molybdic acid. Furthermore, deficient heteropolymolybic ions (PMo_9 species) were also observed. Therefore, an incomplete reaction of phosphoric acid to phosphomolybdic acid is considered. And, PMo_9 is regarded as the intermediate of phosphomolybdic acid.

In the molybdate blue method for phosphate, the complexes are prepared by adding antimonyl potassium tartarate in addition to ammonium molybdate. The solution prepared by this method was measured using ESI-MS. From previous studies, phosphomolybdic acid was the reduced form of $[PSb_2Mo_{10}O_{40}]$ by antimonyl potassium tartarate. But the peaks assigned as this complex were not observed. Nevertheless, the peaks of phosphomolybdic acid were higher than those without antimonyl potassium tartarate. Therefore, the role of antimony was considered to improve the stability of phosphomolybdic acid.

By ESI-MS and solution improvement, new information on complexation reaction process of silicomolybdic and phosphomolybdic acids could be obtained in this study.

Poster Session

Tuesday, 18th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PTu-201

11:10 – 12:20

The Development of Analytical Method for Speciation of Transition Metal Ions in Seawater by CE-ESI-MS

Daisuke Nakamoto, Masashi Wakasugi, Miho Tanaka

Tokyo University of Marine Science and Technology, Tokyo, Japan

Keywords:
CE-MS, transition metals, speciation, seawater

Novel aspects:
For seawater analysis, the separation of trace metal ions from high concentration of NaCl in keeping with the dissolution states of the original metal ions by CE-MS was accomplished.

Abstract:

Introduction

It is difficult to determine concentration of transition metals dissolved in seawater. Acquisition of their dissolution states is more difficult than their determination. In order to characterize transition metal ions in seawater, a new analytical system should be required. ESI-MS (Electrospray Ionization Mass Spectrometry) is known to be one of the soft ionization methods, and many reports on the dissolution states have been presented. However, detection of these dissolution states in seawater is difficult as well as avoiding the "ion-suppression effect ". A high concentration matrix (sodium ion) suppresses detection of trace metal ions. To resolve this problem, pretreatment is required to separate trace metal ions from high concentrations of matrices while keeping dissolution states of original metal ions. In this study, capillary electrophoresis (CE) was used as a separation technique, and online hyphenated CE (-ESI) -MS was developed. The separation method with CE is simple, while it reasonably maintains the dissolution states of objective metals, compared with other separation methods. For CE-MS analysis, volatile background electrolytes (BGEs) are required to obtain high sensitivity. In this study, non-volatile hydrochloric acid (HCl) was used as the BGE because volatile BGEs, such as formic or acetic acids, easily coordinate with transition metal ions. Manganese (II) and Cobalt (II) , which have analogous properties, were measured in high concentrations of NaCl solutions by CE-MS.

Application

CE-MS is composed of online hyphenation of CAPI-3300 (Photal) and LC-MS 2010A (Shimadzu) with self-designed tri-axial ESI interface. Separation voltage (+30kV) was applied by CAPI-3300, and ionization voltage as +4.5kV by LC-MS 2010A. Ultrapure water (18.2MΩ) was used as sheath liquid with 0.01 mL/min flow rate. The uncoated separation capillary (I.D. 70μm, length 110cm) was used.

Experimantal

All sample reagents (Kanto Chemical Co) used are of special grade. 1) Solutions containing 0.1mM of manganese chloride ($MnCl_2$) or cobalt chloride ($CoCl_2$) were measured by ESI-MS. 2) Solution containing 5 mM of $MnCl_2$, $CoCl_2$ and 500mM of NaCl was measured by CE-MS with 1 mM of HCl as BGE. The concentration of 500mM NaCl is almost the same as that of seawater. Conditions of sample injection to separation capillary were examined (5 kPa for 5~120 sec) .

Results and Discussion

1) Measuring solutions of $MnCl_2$ and $CoCl_2$ with ESI-MS

When an aliquot of $MnCl_2$ solution was measured by ESI-MS, $[Mn(OH)(H_2O)_{0\text{-}5}]^+$, $[MnCl(H_2O)_{3\text{-}4}]^+$, and $[Mn(H_2O)_{3\text{-}5}]^{2+}$ were observed. It is previously reported that $[M(OH)(H_2O)]^+$ (M : Metal) is derived from hydrolysis of $[M(H_2O)_2]^{2+}$ at liquid-phase and gas-phase in ESI-MS. When $CoCl_2$ solution was measured, $[Co(OH)(H_2O)_{0\text{-}5}]^+$, $[CoCl(OH)_{3\text{-}4}]^+$ were also observed but $[Co(H_2O)_n]^{2+}$ was not observed. Cobalt ion is more easily hydrolyzed than manganese ion. Thus, accurate information on sample solutions could be obtained by ESI-MS.

2) Measuring solutions of $MnCl_2$ and $CoCl_2$ in high concentration of NaCl with CE-MS

First, amounts of sample solution for the capillary were examined. When injection times of sample solution were 30~60 sec, sharp and high peaks of objective metal species were observed on the electroferrogram. When samples contained extremely high concentration of components, stacking of minor component occurred in the CE system (Self-stacking) . When injection time of sample solution was over 80 sec, cluster ions of NaCl (m/z 81+58n) were also stacked and led to ion-suppression effect. Therefore, the optimal condition of sample injection was 5 kPa for 30~60 sec. Observed metal species with CE-MS were good accordance with ESI-MS. Thus, the separation of trace metal ions from high concentration of NaCl in keeping with the dissolution states of the original metal ions by CE-MS was accomplished.

PTu-202

13:30 – 14:40

Identification of Silk Proteins Excavated from the Ruin of the Makimuku Site in 3-4 AD Japan by MALDI Mass Spectrometry

Kazuki Kawahara[1], Miho Muguruma[1], Teruhiko Hashimoto[2], Kaoru Terasawa[2], Atsuko Miyaji[1], Takashi Nakazawa[1]

[1]Nara Women's University, Nara, Japan, [2]Research Center for Makimuku-Gaku, Nara, Japan

Keywords:
MALDI mass spectrometry, Silk fiber, Sericin, Fibroin, Archaeology

Novel aspects:
Mass spectrometry has proved effective to identify silk proteins in archaeological textile materials even if they are heavily contaminated and degradated.

Abstract:
INTRODUCTION : Very few proteins remain intact even in several days of storage unless some care is taken for preservation. It is therefore extremely difficult to detect a protein supposedly contained in materials of archaeological interest. A few exceptional proteins include fibroin and sericin constituting silk textile, which represents one of the key materials associated with ancient culture and civilization, although these proteins usually suffer from severe biological degradation. The species of a silk moth producing the silk proteins have been identified by optical or electron microscopic observation of characteristic appearance of the threads as far as the morphology is preserved in good condition whatever old the specimen might be. Infrared (IR) spectroscopy can also be used to distinguish between silk proteins and other fibrous materials of plant origin if their molecular structures are not seriously damaged and the level of contamination is reasonably low. However, it is desirable to identify the silkworm even when the specimen is available only a very small amount and severely contaminated with other proteins. In the present study, we suggest a new methodology based on mass spectrometry and proteomics to identify silk fiber. We applied this method on a drawstring pouch recently excavated from the Makimuku site situated in Nara Prefecture, which covers almost the entire capital area of ancient Japan during the last stage of the Yayoi period (about 300 BC to 300 AD) to the early Tumulus period (about 300 AD to 400 AD) .
METHODS : A small fragment (about 1 mg) of drawstring pouch was washed with pure water and triturated manually with a saturated solution of LiSCN to dissolve sericin and fibroin inclusively. The supernatants obtained by centrifugation at 14,000*g* for 15 min were dialysed five times against 25 mM NH_4HCO_3 solution for 48 h. As a reference, solutions of sericin and fibroin were independently prepared from cocoon shells of silkworm *Bombyx mori* (*B. mori*) . These samples were then digested with trypsin at 37°C for 24 h and the digests were analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry using CHCA as a matrix.
RESULTS : The MALDI-TOF MS spectra of tryptic digests prepared from the small fragment of drawstring pouch indicate that the material is highly contaminated with an overwhelmingly large number of contaminant peaks originated from human keratins, microbial proteins, and synthetic polymers such as $(CH_2\text{-}CH_2\text{-}O)_n$- characterized by a series of peaks evenly spaced by 44 Da. Nevertheless, we could identify several peaks with the *m/z* values identical to those of the corresponding peptides of silk proteins (sericin 1, sericin 3, and fibroin) prepared from cocoon shells of silkworm *B. mori*. In particular the detection of the peak at *m/z* 1497 (assigned to QSNTNYADQNSVR) excludes the possibility that the peptide originates from the wild-type species *B. mandarina.* Note that fibroin was detected, but with some difficulty, along with sericin-1 and -3, while this protein was missing altogether in silk fibres embedded in the debris of lacquer-coated coffin of 7th century Japan. This implies that fibroin is much more susceptible to degradation, probably by bacteria, than sericin for more than 1000 years' burial in the soil. Such bacteria include *Variovorax paradoxus*, which can selectively use fibroin as a sole nutrient for growth (Forlani, G., et al., 2000) . Although extending the research to the contemporaneous silk textiles together with reference silk fiber other than *B. mori* and *B. mandarina* would be necessary, we demonstrated that our methodology shown here will provides a clue to investigating the relationship between the sociological value of silk and sericulture in ancient culture and civilization worldwide.

Oral Session

Wedensday, 19th September

Main Hall

09:00 – 11:00

Session 21: Platform Technology for Metabolomics

Chair: Yoshiya Oda (Eisai Inc, USA)

S21-0900

09:00 – 09:40

[Keynote Lecture] New generation high-field Orbitrap instrumentation in untargeted metabolic profiling

Annie M Evans, Qiang Liu, Brandi Bridgewater, Matthew Mitchell, Hongping Dai, Corey DeHaven, Sandy Stewart

Metabolon, Durham, NC, USA

Keywords:
Metabolomics, UPLC/MS, Orbitrap Elite, Q-Exactive

Novel aspects:
The application of the new generation Orbitrap instrumentation, OrbiElite, and Q-Exactive, to untargeted metabolic profiling.

Abstract:
Untargeted metabolic profiling requires identification and quantification of as many metabolites in a biological system as possible, by which the cellular outcomes of some test perturbation, examples, individual genotype, proteomic variation, and toxin exposure, can be revealed. Due to the diversity of metabolites and dynamic range, untargeted metabolic profiling in a high-throughput manner remains a challenge. The recent advances in high-resolution MS instrumentation incorporate desirable features, including fast spectral acquisition rate and high mass accuracy. In this study, the recently introduced Orbitrap Elite and Q-Exactive both equipped with UPLC are evaluated for high-throughput untargeted metabolic profiling. Extracted and vacuum-dried human plasma and/or urine samples were reconstituted with a solution of mixed internal standards in metabolic profiling experiments, or reconstituted with serial-diluted standard solutions for examining detection sensitivity and dynamic range. The Orbitrap Elite and Q-Exactive were equipped with Waters Acquity UPLC. These instruments alternated between MS and data dependent MS/MS scans. Samples were eluted with a 7-min gradient from BEH C18 columns (2.1×100mm, 1.7 μm) and ionized using electrospray/heated electrospray. Data were processed with Xcalibur and in-house software. The key features of the Orbitrap Elite and Q-Exactive and the resulting advantages utilized in untargeted metabolic profiling have been characterized and reported below. The spectral acquisition rate of both the Orbitrap Elite and the Q-Exactive was significantly increased in comparison to its predecessor, the LTQ Orbitrap. This ensured improved data points across a typical chromatographic peak (3-8 s at baseline) for the UPLC system as well as an increase in the number of MS/MS fragmentation spectra obtained. Meanwhile, similar mass accuracy (< 5 ppm) and precision were achieved with Orbitrap Elite, Q-Exactive and LTQ Orbitrap based on the measurement of internal standards and endogenous metabolites in human urine samples. The high-resolution and accurate mass capabilities of the Orbitrap instruments reduced background thus leading to more than a magnitude improvement in lower limit of detection when compared to a low-resolution mass spectral analysis. A linear response range over 3-4 orders of magnitude was demonstrated for the Orbitrap Elite and the Q-Exactive in our study. The detection reproducibility of the Orbitrap Elite and the Q-Exactive was revealed with relative standard deviation (RSD) of peak area for internal standards (<10% in positive mode and <15% in negative) in pooled plasma and urine, which demonstrated an improvement in RSD when compared to similar low-resolution mass spectral analysis. The average number of qualified extracted-ion-chromatographic (XIC) peaks detected per urine sample injection increased nearly 4 fold using the Orbitrap Elite and Q-Exactive. This suggests a potential increase in the number of compounds detected in metabolic profiling. As demonstrated by the peak integration of N-acetylhistidine in individual urine samples, quantification of metabolites (thus the statistic output) was also improved i.e. made more accurate, by minimizing the mass interference in XIC.

Oral Session

Wedensday, 19th September

Main Hall

09:00 – 11:00

Session 21: Platform Technology for Metabolomics

Chair: Yoshiya Oda (Eisai Inc, USA)

S21-0940

09:40 – 10:00

Improving comprehensive analysis for myxobacterial secondary metabolome mining

Thomas Hoffmann[1,2], Daniel Krug[1,2], Rolf Mueller[1,2]

[1]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbruecken/Germany, [2]Pharmaceutical Biotechnology, Saarland University, Saarbruecken/Germany

Keywords:
secondary metabolites, screening, statistics, LC-MS/MS

Novel aspects:
Identification of pharmaceutically relevant compounds using in-house database queries and statistical evaluation of complex bacterial extracts. MS^2 precursor selection based on statistics improve finding of new compounds in screening projects.

Abstract:
As stated by many researchers, extracting relevant information from complex data sets is an important bottleneck in (microbial) metabolomics research. Compared to a focused targeted approach this is even more important in untargeted metabolomics, aiming for the identification of all compounds produced by a particular bacterial strain. Many of these compounds are part of primary metabolism and therefore out of scope when research concentrates on pharmaceutically active secondary metabolites. Other metabolites may be very common for a certain genus of bacteria and already well known but the large subset of compounds that is "really new " is hard to identify.

By combining targeted and untargeted metabolomics approaches it is possible to narrow down/identify numerous features derived from HPLC-high resolution MS/MS measurements of highly complex bacterial extracts. An automated feature finding algorithm extracted around 3000 5000 features within one HPLC-MS chromatogram. In a first step these features were subjected to a search against an in-house database using accurate mass, isotope pattern fit, and retention time in order to identify already known compounds. Untargeted metabolite profiling, using statistical methods such as t-test and PCA analysis could identify features related to the growth of the bacteria by comparing myxobacterial extracts to blank samples (growth medium) . These features were added to a scheduled precursor list (SPL) to focus fragmentation experiments to this relevant subset of compounds within the complex mixture. This enabled a fragmentation of even low abundant features which might have been missed during an automatic precursor selection without predefined compounds of interest. High resolution full scan MS and MS/MS spectra were used to identify target metabolites by queries in an in-house database to identify putative derivatives of known compounds based on a similar fragmentation pattern. The combination of this methodology with genome based metabolome mining approaches further improves the classification of new non-ribosomally synthesized peptide compounds in an early state of research. The whole process covers the very first identification of new secondary metabolites up to the elucidation of the structure and the biosynthetic pathway to end up with final bioactivity studies.

Oral Session

Wedensday, 19th September

Main Hall

09:00 – 11:00

Session 21: Platform Technology for Metabolomics

Chair: Yoshiya Oda (Eisai Inc, USA)

S21-1000

10:00 – 10:20

Understanding alterations of platelets metabolism during storage by using UPLC-Q-TOF-MS strategy.

Giuseppe Paglia[1,2], Olafur E Sigurjonsson[3], Manuela Magnusdottir[2], Steinun Thorlacius[2], Sveinn Gudmundsson[3], Bernhard O Palsson[2], Ines Thiele[2]

[1]University of Iceland, Reykjavik, Iceland, [2]Center for Systems Biology, University of Iceland, Reykjavik, Iceland, [3]The Blood Bank, Landspitali-University Hospital, Reykjavik, Iceland

Keywords:
Metabolomics, Platelets, HILIC, UPLC-Q-TOF

Novel aspects:
To date this is the first metabolomics study on platelets, and it provides understanding of metabolic changes occurring during platelets concentrates storage in transfusion medicine.

Abstract:
Platelets are small anucleate cells having an important role in thrombosis, haemostasis, inflammation, and wound healing. For many years, it was thought that the majority of platelets proteins were synthesized by megakaryocyte and acquired from them during their maturation. Recent evidence has shown that de novo protein synthesis occurs in mature platelets. Consequently, platelets appear to be metabolically more active than previously thought.
Platelets are routinely stored and used in transfusion medicine for clinical purpose. However the storage time is limited (5 to 7 days), because of biochemical and structural changes occurring in platelets during the storage, named platelet storage lesions.
Metabolic profiling provides a global picture of the metabolome by maximizing the number of detected metabolites without *a priori* knowledge of the metabolites present in a sample. The complete set of all metabolites formed by a cell is defined as the metabolome and it comprises the endo-metabolome (all intracellular metabolites) and the exo-metabolome (all the metabolites secreted into the extracellular fluid). During cell culture, continuous exchange of metabolites occurs between the extracellular and intracellular environment, as cells take nutrients up and secrete metabolites. This exchange of metabolites between intracellular and extracellular environment together with the intracellular metabolite profiling provide a complete picture of the metabolism of cells in culture.
In this study we have used an UPLC method (HILIC) coupled with a Q-TOF mass spectrometer (Synapt G2, waters) to study metabolomic changes occurring in the intracellular and extracellular environment of platelets concentrates during their storage.
Three platelet units, each one derived from 5 blood donors (15 different individuals), were stored for 10 days at 22° C under gentle agitation. Samples were collected at day 0, 2, 4, 6, 8 and 10, cells and medium were separated by centrifugation, and extracted with 0.5 mL of methanol : water (7 : 3) and 0.5 mL of acetonitrile, respectively.
During storage, platelets concentrates are maintained in a saline solution together with carry over plasma (between 20 to 30%) at 22° C. Carry over plasma provides nutrients to sustain platelets metabolism.
Significant changes of the exo-metbolome composition over the time were found during the analysis. For instance, platelets use as main fuel glucose and glutamine and secrete large amount of lactate. Moreover, during the storage, platelets take citrate up, which is present in the platelets storage solution at high concentration as chelating agent. Aconitic acid instead resulted increased in the supernatant, suggesting secretion of this metabolite.
Accumulation in the supernatant of intermediates of purine metabolism, xanthine and hypoxanthine, was also found in samples.
On the other hand inside the cells, it was found accumulation of citrate, pyruvate and lactate, while level of aconitic acid resulted depleted over the time. Intermediates of glycolysis seem to be nearly constant over the time.
The changes in the exo- and endo-metabolome composition suggest that storage affects the TCA cycle. The cells continuously metabolize glucose, and pyruvate is mainly converted in lactate, instead of feeding the TCA cycle, which shows a reduced metabolic activity.

Oral Session

Wedensday, 19th September

Main Hall

09:00 – 11:00

Session 21: Platform Technology for Metabolomics

Chair: Yoshiya Oda (Eisai Inc, USA)

S21-1020

10:20 – 10:40

Neuroactive Steroids in Plasma Measured by a Novel Mass Spectrometry Platform

Andre Kopoyan, Karin M Green, Kristina M Deligiannidis, Scott A Shaffer

University of Massachusetts Medical School, Worcester, MA, USA

Keywords:
steroids, quantitation, electrospray, LC-MS, metabolites

Novel aspects:
A nanoflow LC-MS/MS assay for neuroactive steroids capable of detection limits similar to those found by GC-MS methods.

Abstract:
Neuroactive steroids (NAS) have been implicated in a variety of disorders including epilepsy, multiple sclerosis, neurodegenerative diseases, traumatic brain injury, depression, schizophrenia, alcohol dependence, pain and anxiety disorders. Several neuroactive steroids are potent modulators of the $GABA_A$ receptor and consequently can alter the excitability of the central nervous system. Measurements of these analytes are most commonly approached by gas-chromatography mass spectrometry (GC-MS) methods requiring chemical derivatization to decrease polarity and increase sample volatility. Our motivation was to develop an LC-MS/MS method to quantify neuroactive steroids that precluded the need for chemical derivatization and minimized the steps involved with sample preparation. Here we present a novel non-derivatizing LC-MS/MS assay to screen a panel of eight neuroactive steroids in plasma from women classified either as low risk (LR) or high risk (HR) to postpartum depression (PPD) during the perinatal period. The feasibility study measured plasma concentrations from six cohorts from each group and were measured at three gestational age time points and 3 - 9 weeks post-partum.

The assay set out to measure plasma concentrations of progesterone, deoxycorticosterone, 5 a-dihydroprogesterone, 5 b-dihydroprogesterone, allopregnanolone, allotetrahydroxycorticosterone, pregnanolone, and pregnenolone, in addition to GABA (via additional LC-MS/MS assay) . The LOQ for progesterone was determined to be ~200 pg/mL, comparable to the ~100 pg/mL value commonly reported for GC-MS. Preliminary data suggest that allopregnanolone, a potent modulator of the $GABA_A$ receptor, is elevated for the HR over the LR group : 61.2 versus 13.7 ng/mL for the <36 hours cohort, respectively. For GABA, increasing GABA levels were positively correlated to gestational age and into the postpartum (p=0.0029) . Further, HR subjects had an average GABA level 0.34 μg/mL lower than LR subjects (p=0.78) . Results for the NAS pilot study will be presented along with a discussion of measuring neuroactive steroids by LC-MS/MS approaches.

Neuroactive steroids were extracted from human plasma and analyzed against a calibration curve prepared from human charcoal-stripped plasma over the 200 pg to 0.1 microgram per mL range. Samples and calibrants (300 uL) were both spiked with deuterium labeled analogs and processed through a two-step procedure using C18 followed by aminopropyl SPE cartridges. Final eluates were resuspended and injected onto a Waters NanoAcquity UPLC fitted with a 2 cm C18 trap column configured to a 20 cm long (180 um ID) C18 fused silica capillary column with gravity-pulled tip. Neuroactive steroids were eluted using a fast gradient followed by an isocratic elution at 95% methanol. Multiple reaction monitoring was performed using a Waters Quattro Premiere XE operating in the positive ion electrospray mode ; data was processed further by QuanLynx.

Oral Session

Wedensday, 19th September

Main Hall

09:00 – 11:00

Session 21: Platform Technology for Metabolomics

Chair: Yoshiya Oda (Eisai Inc, USA)

S21-1040 Metabolomics Studies Using a Versatile Microfluidic Platform

10:40 – 11:00

Steven A Cohen, Giuseppe Astrarita, Giorgis Isaac, Angela Doneanu, Jay Johnson, Jim Murphy
Waters Corporation, Milford, USA

Keywords:
Metabolomics, microfluidics, lipidomics

Novel aspects:
A microfluidic platform suitable for analyzing small molecules was developed for the analysis of metabolomics and lipidomics samples. This versatility is unprecedented in a microfluidic LC-MS system.

Abstract:
One of the fastest growing "'omics " applications today is the broad based screening of small molecules in biological systems known as metabolomics. Because metabolite studies encompass a diversity of analyte types and a wide dynamic range of concentrations, comprehensive analysis challenges both the chromatographic separation and the mass spectrometry.

This presentation will describe novel microfluidic devices optimized for LC-MS analysis of small molecules and applied to the analysis complex sample mixtures such as those analyzed in metabolomics and lipidomics studies. These devices are fabricated from ceramic materials that permit operation at high pressure with sub 2 micron particles and thus are capable of producing highly efficient UHPLC operation. By optimizing the channel design, and incorporating an integral mass spectrometer emitter, on-board column heating and flexible column formats, the devices are suitable for analyzing a variety of small molecule samples with performance comparable to that achieved with analytical scale LC-MS analysis.

Results : Devices with an internal channel diameter of 150 microns permit no-loss direct injection of microliter sample volumes, with a sensitivity enhancement compared to 2.1 mm ID columns of 20 - 50x. Column efficiency is equivalent to analytical scale chromatography, with peak capacities exceeding 200 within a 10 minute analysis, and greater peak capacities achieved with longer gradients. Typical samples were plasma, urine and brain, heart or liver tissue extracts, and were screened for metabolites by reversed phase and HILIC chromatography. Lipids were analyzed from solvent extracted samples, and amines (including amino acids and biogenic amines) in deproteinized plasma or urine were analyzed after a rapid, simple one step derivatization protocol. Q-Tof and triple quadrupole mass spectrometers were used for untargeted and targeted analyses, respectively. Comparisons with analytical scale columns demonstrated equivalent results, with solvent consumption reduced by > 50 x.

Oral Session

Wedensday, 19th September

Room A

09:00 – 11:00

Session 22: Instrumentation Developments in Mass Spectrometric Imaging

Chair: Anastassios Giannakopulos (ThermoFisher Scientific, Germany)

S22-0900

09:00 – 09:40

[Keynote Lecture] High Resolution in Mass and Space: New Developments and Trends in MALDI Mass Spectrometry Imaging

Bernhard Spengler, Andreas Römpp, Sabine Guenther, Oliver Schulz, Yvonne Schober, Zoltan Takats, Klaus-Peter Hinz, Christian Lotze, Joerg-Ulrich Poetzl, Christian Schinz

Justus Liebig University, Giessen, Germany

Keywords:
Mass Spectrometry Imaging, atmospheric pressure, accurate mass, high spatial resolution, biomolecules

Novel aspects:
Mass spectrometry imaging at a high resolution in mass and space for a high validity of topological analyses of animal and plant tissue.

Abstract:
MALDI Mass Spectrometry Imaging (MSI), 18 years after its first announcement [1], has turned into a routine method of highest performance for the molecular histology of biological tissue [2]. The method, providing high resolution in mass and space, has been developed for a reliable identification and localization of individual tissue components, and has been applied to a number of research areas in pathology, cancer diagnostics, metabolic pathway analysis and plant research. Targeted compound classes include phospholipids, peptides, proteins, drug compounds and metabolites.

An atmospheric pressure ion source was developed in our laboratory, based on a dedicated microoptical setup which was designed for highest optical resolution under mass analytical sampling conditions, providing a spatial resolution on tissue of 3 to 5 micrometers. The ion source was coupled to orbital trapping and ion cyclotron resonance analyzers, at a mass accuracy of better than 1 ppm RMS. Ion images were acquired with an acquisition speed of more than 2 pixels per second in asynchronous and about 1 pixel per second in synchronous sampling mode.

The presentation will describe technical principles of the new instrumentation in comparison to alternative approaches described in the literature, including optical focusing, ion formation and transfer at atmospheric pressure, image acquisition and substance identification. It will also include examples for biomolecular imaging of phospholipids [2], peptides [3] and drug compounds [4] in mammalian, insect and plant tissue at high spatial resolution, confirmed by MS/MS imaging experiments and histological staining of measured samples. Tumor tissues were characterized by their phospholipid patterns, providing structural features invisible in histological staining experiments. Phospholipids are less specific than proteins, but their pattern can be used to distinguish different tumor types and also to distinguish primary tumors from their metastases.

Direct detection and identification of proteins on tissue is limited by a number of factors including limited mass range and fragmentation efficiency as well as incompatibility with formalin-fixed samples. On-tissue digestion of proteins and detection of the resulting peptides can overcome some of these limitations. In a current study we used a newly developed spraying device to apply trypsin on tissue sections [5]. With this setup we were able to obtain a spatial resolution of 50 µm for tryptic peptides.

Combining high spatial resolution down to 3 µm with high mass resolution, high mass accuracy, high imaging selectivity, MS^2 capability and an improved sample preparation technology resulted in a significantly improved quality of MS imaging data in our experiments. One of the main conclusions from the results is that imaging mass spectrometry of biological tissue in general requires highly accurate raw data from FT instruments, since low accuracy data were found to contain a high risk of creating ambiguous or invalid information in imaging experiments of complex biological tissues.

Acknowledgements
Financial support: State of Hesse (LOEWE Research Focus 'AmbiProbe'), European Union (STREP project COMPUTIS), European Reseach Council (ERC Starting Grant Zoltan Takats), and Deutsche Forschungsgemeinschaft (DFG, Sp314/10-1, 12-1, 13-1).

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Oral Session

Wedensday, 19th September

Room A

09:00 – 11:00

Session 22: Instrumentation Developments in Mass Spectrometric Imaging

Chair: Anastassios Giannakopulos (ThermoFisher Scientific, Germany)

S22-0940

09:40 – 10:00

Development and Applications of New Stigmatic Mass Microscope with High Mass and Spatial Resolving Power using Multi-Turn Time-of-Flight Mass Spectrometer

Jun Aoki, Hisanao Hazama, Kunio Awazu, Michisato Toyoda

Osaka University, Osaka, Japan

Keywords:
Imaging mass spectrometry

Novel aspects:
We developed new stigmatic imaging mass spectrometer and achieved mass resolving power of 10000 and spatial resolution of 1 micron. Preliminary data of some applications were also obtained.

Abstract:
Measurement methods of spatial distribution of molecules such as proteins and drugs at cellular-scale are required in many fields including pathology, pharmacology, etc. Recently, sanning type imaging mass spectrometry (IMS) with matrix-assisted laser desorption/ionization (MALDI) is intensively used for biomolecular analysis. However, the spatial resolution of sanning MALDI-IMS is limited by the laser focus diameter to about 10 - 100 μm and inadequate for cellular-scale observation. Therefore, we are developing a stigmatic MALDI imaging mass spectrometer, in which spatial resolution of sub-micron can be achieved irrespectively to the laser focus diameter. We developed a new imaging mass spectrometor to improve measurement performance.

The experimental apparatus consists of a matrix-assisted laser desorption/ionization (MALDI) ion source, a multi-turn time-of-flight mass spectrometer (MULTUM-IMG) and a time and position sensitive delay line detector. The components of the ion optical system were designed and assembled to achieve high accuracy alignment. Ion distributions at the sample plate are magnified and projected with the ion optical lens system onto the detector. MULTUM-IMG which has four toroidal sector electric fields constitute a figure-eight trajectory is inserted into the ion flight path. The ion optical system of MULTUM-IMG satisfies the perfect spatial and temporal focusing condition, so that the spatial distributions of ions before entering the multi-turn circuit are conserved after circulation.

A result of experiment carried out with our prototype apparatus indicate that an alignment accuracy of the ion optical system is critically important to obtain high quality ion image. Thus we designed new optical system as high rigidity block component to be assembled with high accuracy. Main components of the optical system are fabricated with titanium for high rigidity. We observed a dried droplet of crystal violet dye solution covered by a metal foil with fine slits of 1 micro-meter width for evaluating performance of our new apparatus. The slits were formed by fast ion beam etching. As a result, ion image of the sample can be observed after 4 cycles in MULUM-IMG with appropriate voltage applied to electrodes consistent with the theoretical value. The flight path length of ions reached 6.2 m. We also evaluated a new ion extraction method optimum for a stigmatic IMS. Ion distribution is not conserved with ordinary delayed extraction method. By using post extraction differential acceleration (PEDA) method, time focusing and image conservation can be simultaneously satisfied. Our evaluation experiment demonstrated that both the stigmatic observation of ion images with spatial resolution of 1 micro-meter and the mass resolving power of 10000 was simultaneously achieved.

We applied this new apparatus to several practical applications ; i.e. observation of sustance distribution in biological tissue and fine structure of highly-functional organic material. Preliminary data of these experiments are reported in presentation.

Oral Session

Wedensday, 19th September

Room A

09:00 – 11:00

Session 22: Instrumentation Developments in Mass Spectrometric Imaging

Chair: Anastassios Giannakopulos (ThermoFisher Scientific, Germany)

S22-1000

10:00 – 10:20

An Evolution of TOF-SIMS for Biological Samples: From 2D Imaging to 3D FIB-TOF Tomography

John S Hammond, Gregory L Fisher, Scott R Bryan

Physical Electronics, Chanhassen, Minnesota, USA

Keywords:
Imaging Mass Spectrometry, FIB-TOF-SIMS

Novel aspects:
3D TOF-SIMS Tomography of organic and inorganic materials with chemical and molecular sensitivity, < 0.5 micron spatial resolution and depth of analysis > 10 microns

Abstract:
TOF-SIMS has emerged as an important tool for 2D and 3D imaging mass spectrometry of biological and complex material specimens due to it's unique capability to detect molecular and elemental ions at a spatial resolution of < 0.3 μm with a high mass resolution of ~ 15,000 m/Δm without the sample treatments required by e.g. MALDI or fluorescence microscopy. For many biological and materials specimens, TOF-SIMS also has the capability to image samples having a large degree of surface topography. The resulting elemental and molecular images provide important information regarding the composition of biointerfaces, tissues and cells, as well as many complex structures such as solid oxide fuel cells and OLEDs.
An example of the high spatial resolution 2D chemical imaging capability of TOF-SIMS will be discussed to provide the differentiation of epicuticular components present at the surfaces of *Arabidopsis thaliana* organs. The mass spectra were acquired with an analytical sampling depth of ~ 2 nm. Mass spectra acquired from specialized cells forming the stomates and trichomes reveal that, even for single cells on the surface of an organ, the wax composition may vary.
To extend the TOF-SIMS chemical imaging and molecular characterization deeper than the outer 2 nm of the sample, cluster ion beams have been developed to remove multiple layers of material between analysis (chemical imaging) cycles. Examples of this approach to provide 3D reconstructions of tissue samples and cells to a depth of 1 µm will be discussed. Nevertheless, there are practical limitations of the use of ion beam sputtering for probing both organic and inorganic specimens beyond the surface region. Accumulated ion beam-induced damage may occur and give rise to incorrect chemical distributions. Certain matrix components may sputter at a different rate than others, called preferential or differential sputtering, which results in a distortion or complete loss of the true 3D chemical distribution as a function of sputtered depth. Finally, many specimens contain void spaces that are impossible to preserve in 3D images obtained by sputter depth profiling.
An alternative approach to achieve 3D chemical imaging of chemically complex and porous specimens is to utilize *in situ* FIB milling and sectioning in conjunction with TOF-SIMS chemical imaging what we have called FIB-TOF tomography. With FIB milling, the interior of a specimen is revealed to depths of more than 100 μm. 3D chemical imaging with a 3D spatial resolution < 0.5 µm and with a z-dimension of greater than 10 μm may be achieved within a reasonable analysis time. The advantage of the FIB-TOF approach is that artifacts caused by sputter depth profiling, such as differential sputtering and accumulated ion beam damage to matrix molecules, are avoided. Examples of 3D FIB-TOF tomography will be illustrated and discussed for drug distributions in biological applications as well as for complex inorganic samples.

Oral Session

Wedensday, 19th September

Room A

09:00 – 11:00

Session 22: Instrumentation Developments in Mass Spectrometric Imaging

Chair: Anastassios Giannakopulos (ThermoFisher Scientific, Germany)

S22-1020

10:20 – 10:40

Study of Toxin Distribution in Sprouted Potatoes by Electrospray Laser Desorption Ionization Imaging Mass Spectrometry (ELDI/IMS)

Yichia Huang[1], Min-Zong Huang[1], Yi-Tzu Cho[2], Jentaie Shiea[1]

[1]National Sun Yat-sen University, Kaohsiung, Taiwan, [2]Yuh-Ing Junior College of Health Care & Management, Kaohsiung, Taiwan

Keywords:
Electrospray laser desorption Ionization, Imaging mass spectrometry, glycoalkaloids

Novel aspects:
Using electrospray laser desorption ionization imaging mass spectrometry to study of toxin distribution in sprouted potatoes.

Abstract:
As an ambient ionization source, electrospray laser desorption ionization mass spectrometry (ELDI/MS) has been demonstrated to be useful in detecting proteins and small organic compounds on the solid without sample pretreatment. The analyte molecules were desorbed using a pulse laser then post-ionized in an ESI plume. With the assistance of the precise movement of a stepper motor and laser desorption for sampling at high spatial resolution, it is possible to obtain the information of predominant chemical compounds from a particular sample surface via ELDI/MS, and makes it become a promising analytical tool to perform ambient imaging analysis on tissues.
When exposing potato tubers under sunlight, it has known that sunlight triggers the production of specific toxin glycoalkaloids. This is a natural defense mechanism for preventing the uncovered tuber from being consumed by animals. The distribution of glycoalkaloid toxins on the surface of a sprouted potato was examined in this study via ELDI/MS analysis, and the best glycoalkaloid ion signals were obtained. The ELDI imaging of potato slices indicated that the majority of toxins was detected in the cortex and the amount of the toxins decreases with the distance from the potato skin. In addition, more glycoalkaloids were detected as the potato was exposed to sunlight for a longer time. It was also found that different types of the toxins were produced at the cortex part of potatoes near the skin and sprouting buds.

S22-1040
10:40 – 11:00

Conventional and stigmatic laser desorption/ionization-imaging mass spectrometry applied to analyses of organic layers in electronic devices

Yuko Tachibana[1], Yoji Nakajima[1], Tsuguhide Isemura[1], Kiyoshi Yamamoto[1], Takaya Satoh[2], Jun Aoki[3], Michisato Toyoda[3]

[1]Asahi Glass Co., Ltd., Yokohama, Japan, [2]JEOL Ltd. , Akishima Tokyo, Japan, [3]Osaka University, Osaka, Japan

Keywords:
Imaging mass spectrometry, Laser desorption/ionization, High-resolution time-of-flight mass spectrometry, Stigmatic imaging mass spectrometry, Electronic device

Novel aspects:
Imaging LDI-TOFMS with high mass resolution was shown to have potintial to be a powerful analytical technique for application to electronic devices such as OLED.

Abstract:
In recent years, the electronic devices composed of organic and inorganic materials such as organic light-emitting diodes (OLED) and organic semiconductor devices have been widely used. These organic materials are often easily degradable as compared with inorganic materials. Thus, it is important to analyze the degradation area of organic materials in devices and to understand the degradation mechanism.

There are various techniques for elemental analysis such as electron probe microanalysis (EPMA) , x-ray photoelectron spectroscopy (XPS) and so on. However, the analytical techniques to obtain the molecular information on organic layers in devices are limited and insufficient, typically, time-of-flight secondary ion mass spectrometry (TOF-SIMS) . In TOF-SIMS, molecular ions do not appear because of strong fragmentation in many cases, and the unknown material is not often determined because the accurate mass is difficult to be acquired. Besides, extremely high surface sensitivity sometimes prevent from getting the information of the objective material under surface contamination, because the information of surface contamination constitute the majority and the desired information of organic layer is obscured.

For analyses of organic layers in electrical devices, we investigated to apply laser desorption/ionization high-resolution time-of-flight mass spectrometry (LDI-HRTOFMS) utilizing SpiralTOF-TOF tandem mass spectrometer (SpiralTOF) with the spiral trajectory ion optical system. It might be expected that most of the organic layer materials used in OLED or organic semiconductor devices are ionized by LDI without or less fragmentation, because they have optical absorption in UV region. In addition, SpiralTOF makes possible to obtain the accurate mass of materials, and high-energy collision-induced dissociation (CID) provides characteristic structural information. The information obtained by SpiralTOF and CID can provide us not only molecular weight but also elemental composition information and characteristic structural information. These information could be clarified the phenomena that had occurred in organic layers in electrical devices and the degradation mechanism that suggest to solve various problems. Besides, the ionization depth in LDI is around several tens nanometers, so the information of organic layer is obtained without disturbing that of surface contamination. For these, it could be expected that LDI-HRTOFMS utilizing SpiralTOF has potential to be a powerful technique to analyze organic layers in electronic devices.

We observed the organic layer materials of OLED on glass are desorped and ionized by laser radiation without matrix materials and cation agent. The measured OLED panel was prepared by removing the device sealing and aluminum cathode, and succeeding exposure in air for one year. The organic layer material, *N,N'*-di- 1 -naphthaleyl-*N,N'*-diphenyl-1,1'-biphenyl-4,4'-diamine (NPD) , was measured with accurate mass and several tens of thousands of mass resolutions by direct measurement of glass sample with LDI-HRTOFMS utilizing SpiralTOF.

We also examined imaging mass spectrometry (IMS) on this glass sample with SpiralTOF and obtained a clear image of the degraded area of NPD layer. The spacial resolution was around 20 micron, similar to that of XPS which is widely used in analysis of electrical devices. Furthermore, We also challenged to examine the stigmatic type imaging mass spectrometer using a multi-turn TOF mass spectrometer 'MULTUM-IMG' developed by Osaka University group. The spacial resolution was a few micron and the required time to measure was remarkably reduced.

For these investigations, it can be expected that imaging LDI-HRTOFMS has potential to be a powerful technique to analyze the organic layers in electronic devices, for clarifying the phenomena that occurred in devices and obtaining solution to resolve various problems.

Oral Session

Wedensday, 19th September

Room B-1

09:00 – 11:00

Session 23: Gas Phase Fragmentation Mechanisms of Biomolecular Radicals

Chair: Shigeo Hayakawa (Osaka Prefecture Uiniversity, Japan)

S23-0900

09:00 – 09:40

[Keynote Lecture] Gas Phase Chemistry of Biomolecular Radicals - An Overview

Michael Leeming[1,2], William A Donald[1,2], Richard A J O'Hair[1]

[1]The University of Melbourne Victoria 3010 AUSTRALIA, [2]ARC Centre of Excellence for Free Radical Chemistry and Biotechnology

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Keywords:
radical ; amino acids ; peptides ; nucleic acids ; fragmentation

Novel aspects:
Keynote lecture that provides an overview of the gas phase chemistry of biomolecular radicals

Abstract:
The presence of a radical site opens up new types of gas phase chemistries that are not available for even-electron biomolecular ions. In this keynote lecture, a brief overview is given on the : ways of forming biomolecule radical ions ; types of radical ions that can be formed ; types of radical directed reactions that can occur ; rearrangement of radical sites via intramolecular hydrogen atom transfer or tautomerization reactions. Amino acids, peptides and nucleic acids will be used to illustrate key concepts using examples from our and other laboratories.

S23-0940 Mechanism of "Oxygen rearrangement" in mass spectrometry

09:40 – 10:00

Nino G Todua, Karl K Irikura, Stephen E Stein, Anzor I Mikaia

National Institute of Standards and Technology

Keywords:
Oxygen rearrangement, EI mass spectrometry.

Novel aspects:
Mechanism of "oxygen rearrangement " is presented.

Abstract:
Introduction. Hydrogen migration under electron ionization (EI) was discovered in the late 1940s. Later this process was studied and explained by Happ [1] and Nicholson [2] and subsequently utilized by McLafferty [3]. However the migration of the oxygen atom connected to carbon atom under mass spectrometry conditions was not observed or even thought to be possible. Recently [4] carbonyl oxygen migration has been suggested in the EI mass of N-alkyl-N-arylsulfonylbenzamides. The results of study of this phenomenon will be presented.
Materials and Methods. N-Benzoyl-arylsulfonamides and their ^{18}O-benzoyl-analogs were prepared by reaction of arylsulfonamides with benzoic acid or ^{18}O-benzoic acid in pyridine. The EI mass spectra are recorded on GC-MS systems with quadrupole and magnetic sector analyzers. Exact mass determinations and linked-scan are performed on a double-focusing mass spectrometer. Theoretical calculations employed the B 3 LYP hybrid density functional, together with 6 -31G (d) basis sets. Vibrational zero-point energies were scaled by 0.9757. Stationary points were characterized by Hessian analysis, and the reaction mediated by each transition structure was confirmed by IRC calculations. The standard uncertainty of the calculated energetics is estimated to be 24 kJ/mol.
Results. Mass spectra of mono-, di- and trimethyl-sulfabenzamides (I a-c) revealed an unexpected base peak at *m/z* 118 (ion **a**) ; this peak was shifted by 3 Da from 118 to 121 for N-trideuteromethyl-, N,N'-di (trideuteromethyl) - and N,N',N'-tri (trideuteromethyl) analogs. The exact mass measurements (118.0657) confirmed the molecular formula for the ion **a,** and MS-MS spectra indicated the formation of the ion **a** from $M^{+\cdot}$ and from the radical cation $[M\text{-}SO_2]^{+\cdot}$ All the above supported a formation of the methylbenzonitrilium cation $[C_6H_5CN\ (CH_3)]^+$, which involved a migration of the amidic O-atom. The unsubstituted sulfabenzamide (II) demonstrates a base peak of para-aminophenol cation radical under electron ionization.
Theoretical calculations investigated possible mechanisms of this phenomenon. An *ipso* attack of the amide oxygen upon the aryl sulfonyl moiety had an especially small energy barrier (about 40 kJ/mol for I and 57 kJ/mol for II) . SO_2 is expelled, an entropically favorable process, leading to a carboximidate intermediate. For II, the intermediate dissociates to the thermodynamically favored products : a phenol radical cation (the observed base peak) and benzonitrile. There is a barrier for I, leading instead to kinetic products of simple bond homolysis : a phenoxyl radical and methylbenzonitrilium ion (the observed base peak) .
The theoretical mechanism was supported by subsequent isotopic experiments. Thus, substituted phenol radical cations in the mass-specta of N- (arylsulfonyl) benzamides were shifted by 2 Da in the spectra of ^{18}O-benzamide-analogs. It was concluded that the mechanism involves an *ipso* attack of the amide oxygen.

Literature

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Oral Session

Wedensday, 19th September

Room B-1

09:00 – 11:00

Session 23: Gas Phase Fragmentation Mechanisms of Biomolecular Radicals

Chair: Shigeo Hayakawa (Osaka Prefecture Uiniversity, Japan)

S23-1000
10:00 – 10:20

The Cn Ions Formed by the Dissociation of an N-terminal Deuterohemin Containing Hexapeptide

Xinhua Guo, Bing Wang, Jiayi Yu
Jilin University, Changchun, China

Keywords:
deuterohemin ; peptide dissociation ; cn ions ; tandem mass spectrometry

Novel aspects:
The Cn ions are detected in CID mass spectra of a deuterohemin containing hexapeptiede which involve the McLafferty rearrangement reaction

Abstract:
Gas phase dissociations of an N-terminal deuterohemin modified hexapeptide are studied with an ion trap mass spectrometer. Due to a fixed charge in the N-terminal of the peptide characteristic precursor ions that run short of an added proton in single, double and treble charge states are observed in the mass spectra. Upon collision induced dissociation (CID) the singly charged peptide ion generates b_n+17 (c_n) and b_n+18 ions in addition to b_n and b_n derived product ions while the doubly charged peptide ion forms normal sequencing b and y ions. The formation pathway of the c_n ions is proposed that involves a McLafferty rearrangement reaction in which companying a γH transfer from carbon to carbonyl oxygen a cleavage at N-C_α bond instead of an amide bond occurs to the peptide. In contrast, the formation of b_n+18 ions is attributed to the nucleophilic attack of hydroxyl or carboxyl oxygen in the side chain of an amino acid C-terminal to the cleaved amide bond, which competitively happens prior to the McLafferty rearrangement reaction. Since the c_n ions are normally detected in electron capture dissociation (ECD) or electron transfer dissociation (ETD) fragmentation mode and are seldom observed in CID mass spectra, the factors that lead to the formation of the c_n ions are investigated through replacing the crucial amino acid residues, removing or changing the N-terminal deuterohemin group in the original peptide and analyzing the fragments of resulted peptides. The results provide significant insights into the formation and mechanism of the c_n ions in the specific pepetide upon CID process, alternatively, suggest an easy way to generate c_n ions which will be useful for providing much sequence information to improve peptide and protein identifications.

Oral Session

Wedensday, 19th September

Room B-1

09:00 – 11:00

Session 23: Gas Phase Fragmentation Mechanisms of Biomolecular Radicals

Chair: Shigeo Hayakawa (Osaka Prefecture Uiniversity, Japan)

S23-1020

10:20 – 10:40

Isomerization of Radical Peptide Ions and Subsequent Radical-Induced Dissociations

Ivan Keung Chu

Department of Chemistry, The University of Hong Kong, Hong Kong, China

Keywords:
Dissociation, Isomerization, Peptide Radical

Novel aspects:
Effect of isomerization on radical-induced bond cleavage of peptide ions

Abstract:
Dissociation of peptide radical ions involves competition between charge-induced and radical-induced reactions that can be preceded by isomerization. A better understanding of the interconversions and fragmentations of odd-electron radical peptide ions enrichs the scientific basis underlying peptide dissociation in the gas phase--the underpinning technology in proteomics. The dissociations of peptide radical cations ($M^{\cdot+}$) differ from and complement those of the corresponding even-electron protonated peptide ions of the type $[M + nH]^{n+}$; the latter fragment through amide bond cleavages to give predominantly *y* and *b* ions, while the peptide radical cations undergo peptide backbone cleavages of their N-C_α and C_α-C bonds to give *c*/*z* pairs and *a*/*x* product ions, respectively (*a*, *b*, and *c* ions are N-terminal containing ions, while *y* and *z* ions are C-terminal containing ions) . Despite some recent advances from investigations of the reactivities of radical cationic peptides, their fragmentation mechanisms remain poorly understood. In this talk, we will monitor the effect of a basic amino acid residue on various radicalinduced bond cleavages such as C_α-C and N-C_α backbone bonds as well as C_α-C_β and C_β-C_γ bonds of side chain cleavage. A combination of mass spectrometric experiments and theoretical calculations were used to examine the isomerization of radical peptide cations and their radical-site-specific fragmentations. The laser-induced dissociation (LID) and low-energy collision-induced dissociation (CID) spectra of the radical cationic peptides with well-defined initial locations of the radical centers are significantly different from those of their basic analogs. DFT calculations revealed that the basic radical cations have their charge densities largely sequestered on the guanidine groups of the side-chain arginine residues ; the lower charge densities on the backbones result in greater flexibility, decreasing the barriers for interconversion between these α-carbon-centered radicals ; thus the energy barriers for the isomerization of radical cationic isomers were comparable with those of its competitive fragmentation reactions. It is apparent that the highly basic arginine residue in the peptide radical cations could mediate the facile migration of the various radical centers prior to promoting their subsequent side chain cleavages.

Oral Session

Wedensday, 19th September

Room B-1

09:00 – 11:00

Session 23: Gas Phase Fragmentation Mechanisms of Biomolecular Radicals

Chair: Shigeo Hayakawa (Osaka Prefecture Uiniversity, Japan)

S23-1040 Cascade Dissociations of Peptide Cation Radicals

10:40 – 11:00

Frantisek Turecek

University of Washington, Seattle, WA, USA

Keywords:
cascade dissociations, peptide cation radicals

Novel aspects:
Mechanisms have been elucidated for the consecutive dissociations of peptide cation-radicals using experimental and computational methods.

Abstract:
Amino acid residue-specific backbone and side-chain dissociations of peptide z ions in MS 3 spectra were elucidated for over 40 pentapeptides with arginine C-terminated sequences of the AAXAR and AAHXR type, nonapeptides of the AAHAAXX "AR and AAHAXAX "AR type, and AAHAAXX "AAR decapeptides. Peptide z (n) ions containing amino acid residues with readily transferrable benzylic or tertiary beta-hydrogen atoms (Phe, Tyr, His, Trp, Val) underwent facile backbone cleavages to form dominant z (n-2) or z (n-3) ions. These backbone cleavages are thought to be triggered by a side-chain beta-hydrogen atom transfer to the z ion C-alpha radical site followed by homolytic dissociation of the adjacent C (alpha) -CO bond, forming x (n-2) cation-radicals that spontaneously dissociate by loss of HNCO. Amino acid residues that do not have readily transferrable beta-hydrogen atoms (Gly, Ala) do not undergo the z (n) to z (n-2) dissociations. The backbone cleavages compete with side-chain dissociations in z ions containing Asp and Asn residues. Side-chain dissociations are thought to be triggered by beta-hydrogen atom transfers that activate the C (beta) -C (gamma) or C (beta) -heteroatom bonds for dissociations that dominate the MS 3 spectra of z ions from peptides containing Leu, Cys, Lys, Met, Ser, Arg, Glu and Gln residues. The Lys, Arg, Gln, and Glu residues also participate in beta-hydrogen atom transfers that trigger other side-chain dissociations.

Dissociations of z (4) ions from pentapeptides AAXAR where X = H, Y, F, W, and V produce dominant z (2) ions that account for >50% of the fragment ion intensity. The dissociation has been studied in detail by experiment and theory and found to involve several isomerization and bond-breaking steps. Isomerizations in z (4) ions proceed by amide trans-cis rotations followed by radical-induced transfer of a beta-hydrogen atom from the side chain, forming stable C (beta) radical intermediates. These undergo rate-determining cleavage of the C (alpha) -CO bond at the X residue followed by loss of the neutral AX fragment, forming x (2) intermediates. The latter were detected by energy-resolved resonant excitation collision-induced dissociation (CID) and infrared multiphoton dissociation (IRMPD) experiments. The x (2) intermediates undergo facile loss of HNCO to form z (2) fragment ions, as also confirmed by energy-resolved CID and IRMPD MS 4 experiments. The loss of HNCO from thex (2) ion from AAHWR is kinetically hampered by the Trp residue that traps the OCNH radical group in a cyclic intermediate.

Oral Session

Wedensday, 19th September

Room D

09:00 – 11:00

Session 24: Regulated Bioanalysis

Chair: Shinobu Kudoh (Shimadzu Techno-Research, Inc , Japan)

S24-0900 [Keynote Lecture] Drug regulation and bio-analysis

09:00 – 09:40

Tatsuo Kurokawa
Keio University, Tokyo, Japan

Keywords:
JBF, drug development, regulatory science, international harmonization, guideline

Novel aspects:
Activities of recently formed Japan Bio-analysis Forum will be intrduced. Progress of draft bio-analysis guideline in Japan in JBF will be touched upon.

Abstract:
Approach and pathway of drug development as well as scenes of drug therapy have been dramatically changed since introduction and application of molecular biology and human genomic sciences. At the same time, many countries and regions now realize the absolute and urgent needs of optimizing the use of medical resources in front of economic difficulty and rapid increase of health care demands. Bio-analysis technology is now considered as one of key factors for our societies to find the very best solution upon this almost inconsistent issues. The bio-analysis technology can be a strong tool for providing people with the best recommendable therapy in accordance with his/her particular condition. Appropriate utilization of bio-analysis technology in the drug development stage may allow us to reduce the total time and process for a drug development and it will obviously improve the availability and affordability of such new therapeutic options and proposals for patients. This is one of the important subjects of regulatory science.
A group named Japan Japan Bio-analysis Forum, which consists of regulators, industries and academia, has been formed in 2010 with consideration of progressing international harmonization in this area and it started works for establishment of necessary guidelines in Japan.
The above subjects will be introduced and discussed in this session.

Oral Session

Wedensday, 19th September

Room D

09:00 – 11:00

Session 24: Regulated Bioanalysis

Chair: Shinobu Kudoh (Shimadzu Techno-Research, Inc , Japan)

S24-0940 Bioanalytical method validation: Process of preparation and notable points of the draft Japanese guideline

09:40 – 10:00

Noriko Katori

National Institute of Health Sciences, Tokyo, Japan

Keywords:
Bioanalysis, Bioanalytical Method Validation, Japanese Guideline

Novel aspects:
This presentation is the first announcement of the Japanese BMV guideline draft in English.

Abstract:
The FDA released the Guidance for Industry on Bioanalytical Method Validation (BMV) in May 2001. A draft of the revised guidance is expected later this year. Also EMA released the Guideline on BMV in July2011.
A Draft Japanese Guideline on BMV will be released this year. The history of discussion around regulated BMV and the preparation process of the Japanese BMV guidelines will be presented.

The first symposium for regulated BMV held in Japan was the workshop titled "Discussion on AAPS/FDA White Paper for bioanalytical method validation " planned in The 56th Annual Conference on Mass Spectrometry (Tsukuba, Japan) in 2008, where Dr. Viswanathan (FDA) was invited. After the symposium, the interest to regulated BMV was getting higher in Japan year by year.
The organization of Global Bioanalysis Consortium (GBC) and the First Asia Pacific Conference on Recent Issues in Regulated Bioanalysis (Shanghai, China) triggered a formation of the first and only association for scientific discussion of regulated bioanalysis and BMV in Japan. The group became the Japan Bioanalysis Forum (JBF) which has helped to assign Japanese representatives for all Harmonization Team (HT) in GBC. In spite of the Great East Japan Earthquake in March 2011, a lot of Japanese have applied to the Harmonization Teams of GBC with willingness. At the same time, JBF has created a discussion group to support all Japanese HT members in the GBC.
The first Symposium of JBF was held in August 2011 in Tokyo, and full-fledged discussions of regulated bioanalysis have been started in Japan. The JBF symposia have been held about every six months until now.

Responding to the enthusiastic discussion around BMV, the Japanese regulatory authority prepared a study group in which the BMV has been discussed among regulation sides and companies. The study group is preparing the draft Japanese guidelines on BMV based on the tremendous contributions by JBF. The policy of the preparation of guideline is as follows ;

* Applied to bioanalytical methods generating quantitative data in toxicokinetics and clinical studies.
* Sections for Small molecule are prepared firstly.
* Sections for Large molecule will be following.
* Analysis of metabolite can be considered.
* Analytical methods : Chromatographic methods, e.g. LC-MS, LC-MS/MS.
* Terminology ; conform to ICHQ 2 a, b.
* Aligned with the FDA Guidance /EMA guideline.

I will show you the summary of the Draft Japanese Guideline on BMV.

Oral Session

Wedensday, 19th September

Room D

09:00 – 11:00

Session 24: Regulated Bioanalysis

Chair: Shinobu Kudoh (Shimadzu Techno-Research, Inc , Japan)

S24-1000

10:00 – 10:20

Using of Multistage Mass Spectrometry Techniques in Combinations with Electrophoretic and Chromatographic Separation Techniques for Analysis of Biological Samples

Jozef Marak, Andrea Stanova, Monika Kondekova, Monika Radicova, Nikoleta Biherczova, Katarina Krcova

Comenius University, Bratislava, Slovak Republic

Keywords:
multistage MS, separation techniques, complex biological samples, therapeutic drugs

Novel aspects:
Multistage IT-TOF MS technique was used for the identification of the analytes present in complex samples and for the proposal of their structures and possible changes under in vitro conditions.

Abstract:
Bioanalytically important compounds, such as low- and high-molecular weight compounds naturally occurring in the living beings, or different xenobiotics and/or their metabolites, are often needed to be determined in various biological samples at (ultra) trace concentration levels. Apparently, the complex characters of all biological samples are representing the top challenges for the analytical chemistry technologies used nowadays. For example, consider several hundreds of different constituents as these still spanning various chemical/biological properties and, in addition, concentrations of analytes as differing several orders of magnitude. Due to these facts, a combination of a powerful separation technique is required, especially, to reflect for an adequately sensitive and/or selective detection method. There are two main analytical separation techniques used for solving these problems, liquid chromatography (LC) and capillary electrophoresis (CE) . LC techniques, mainly in nanoLC variation, served as "standard " analytical separation technique due to its "universality " and flexibility. However, its mostly not sufficient separation efficiency and the production of toxic waste are limiting factors for its acceptance as the "universal " analytical separation tool. On the other hand, while CE techniques offer some favorable properties, like very high separation efficiencies along with very low consumption of both the samples and the operating solutions, they suffer from the detection sensitivity. MS detection itself or in the combination with LC or CE techniques provides an excellent selectivity while keeping sufficient detection sensitivity. However, because of the complex character of biological samples and different concentration levels of the analytes of interest and the matrix components even LC-MS and/or CE-MS combinations are not always able to solve successfully the above mentioned analytical problems and some sample pretreatment technique is required to be implemented into the analytical scheme.
The experiments with micropreparative capillary isotachophoresis (pITP) , used in our approach as the sample pretreatment technique, were performed in a hydrodynamically closed CE separation system. Different biological samples (urine, saliva and tear samples served as the model matrices in our case) were used alone and/or spiked with several low- and high molecular weight compounds (model analytes) and pITP served as a tool for isolating analytes while accompanied only by minimum interferents from the biological matrices. Such an isolation procedure was performed with the help of a fractionation valve. pITP was included for discrete spacer constituents as added to the sample solutions. The isolated fractions were lyophilized and then reconstituted for final mass spectrometric analyses by : (i) a direct injection mode, (ii) HPLC-MS (UPLC-MS) and (iii) CE-MS combinations.
Our work deals with different approaches and analytical aspects as regarding low- and high molecular weight compounds containing samples. Multistage MS based on IT-TOF technique was used for the identification of the analytes of interest present in complex samples and for the proposal of their structures and possible changes under in vitro conditions. A very low concentration level of the analyte by using pITP and, subsequently, as loading to chromatography and electrophoresis techniques as coupled to MS. MS and MS^n spectra were obtained from the reconstructed fractions as included both the pITP clean-up effect and its ITP concentration power as regarding the analyte as present in the complex sample at very low concentration levels. This study is showing high potentialities and compatibility of pITP as the sample pretreatment technique before several modes of MS analysis of the complex samples of biological origin.

Acknowledgement:
This work was supported by : the Slovak Research and Development Agency (the project No. VVCE-0070-07) and the Slovak Grant Agency (the project No. 1/1305/12) .

Oral Session

Wedensday, 19th September

Room D

09:00 – 11:00

Session 24: Regulated Bioanalysis

Chair: Shinobu Kudoh (Shimadzu Techno-Research, Inc , Japan)

S24-1020
10:20 – 10:40

Quantitative Analysis of Beta casomorphin-7 by SRM using stable isotope labeled peptide as internal standard

Xiaomin Song[1], Thiri Zaw[1], Ardeshir Amirkhani[1], Andrew Clarke[2], Mark Molloy[1]

[1]APAF Macquarie University, Sydney, Australia, [2]A2 Co Ltd, Australia

Keywords:
SRM, Beta casomorphin, BCM-7, peptide quantitation

Novel aspects:
Our MS based BCM-7 quantitation method is of better specificity and similar level of sensitivity comparing to ELISA for detecting BCM-7 in body fluids.

Abstract:
Beta casomorphin-7 (BCM-7) is a peptide released from beta-casein when bovine milk is digested. The health effects of BCM-7 in the body have been investigated and shown to play a role in the aetiology of some human diseases, including Type 1 diabetes. In 2009 the European Food Safety Commission (EFSA) released a review relating to BCM-7 ; it reported that BCM-7 was an opioid receptor agonist, with the potential to impart activity on a range of tissues including immune and digestive cells. However it concluded that a cause and effect relationship could not be established between the dietary intake of BCM 7 and non-communicable diseases ; owing in part to lack of data on exposure to BCM-7 following the consumption of milk products.

Historically, the quantitation method for BMC-7 lacked either sensitivity or selectivity and the analysis of BCM-7 in body fluid has been difficult. To address this, we developed a SRM based BCM-7 analysis method using stable isotope labeled BCM-7* (H-Tyr-Pro-*Phe-Pro-Gly-Pro-Ile-OH, where *Phe = $^{13}C9$, ^{15}N phenylananine, mass increase 10Da) as internal standard. BCM-7* was spiked into analyte before BCM-7 enrichments. Because they have identical chemical character, simultaneously acquiring SRM data for native BCM-7 (MW=790.4Da) and stable isotope labeled BCM-7* (MW=800.4Da) effectively compensated the sample matrix effect, sample preparation effect and instrument drifting effect. This allows highly sensitive and specific absolute quantitation of the peptide without the need to run standard calibration curve. Our tests showed that BCM-7 in plasma can be accurately quantitated in low ng/ml range.

Human plasma was used as matrix in the method development and validation. However, this method can be applied to other body fluids or milk products after some sample specific sample preparation tests.

Oral Session

Wedensday, 19th September

Room D

09:00 – 11:00

Session 24: Regulated Bioanalysis

Chair: Shinobu Kudoh (Shimadzu Techno-Research, Inc , Japan)

S24-1040
10:40 – 11:00

Accurate Mass MS/MS an Attractive Option For the Quantification of Biotherapeutics in DMPK Studies

Robert S Plumb[1,2], Christopher Evans[3], Paul D Rainville[1], Joanne Mather[1]

[1]Waters Corporation, [2]Imperial College, London, UK, [3]GlaxoSmithKline, DMPK, PA, USA

Keywords:
Bioanalysis, Protein, accurate mass, LC/MS/MS

Novel aspects:
The use of accurate mass LC/MS with narrow mass extraction range to detect and quantify protein therapeutics in plasma extract

Abstract:
In this paper we discuss the application of LC-HRMS for the quantification of a the rapeutics protein. Biotherapeutics now represent approximately20% of a typical pharmaceutical companies drug pipeline, and must be evaluated for safety and efficacy in a similar manner to small molecules. Tandem quadrupole LC/MS/MS is the technology of choice for the quantification of small molecules in DMPK studies. The application of accurate mass MS for quantification in DMPK has attracted considerable interest in recent years, allowing simultaneous quantitative and qualitative analysis. By employing a narrow mass window assay the specificity is significantly improved, increasing the S/N and assay accuracy. The results generated in this study were compared to those previously acquired on a tandem quadrupole MS system.

The plasma samples (50 µL) was digested with an equal volume of Lys-C solution overnight, the sample centrifuged and the resulting solution isolated by means of cation exchange SPE. The resulting sample was evaporated to dryness and then reconstituted in mobile phase starting conditions for analysis by LC/MS. The LC/MS analysis was performed on a hybrid quadrupole TOF mass spectrometer operated in positive ion mode. A the separation was performed on a 2.1 x 5 cm 1.7um C18 column operated in reversed phase gradient mode over 2 minutes. The quadrupole was set to 503.5 the TOF analyser range was 50 1000 m/z. Quantification was performed using the mass fragment m/z = 616.3821, with 510.5 => 623.399 employed for the internal standard.

The two most common approaches for the quantification of biotherapeutic drugs are either the analysis of the intact protein or analysis of a signature peptide following protein enzymatic digest. The use of a proteolytic digestion followed by measurement of a signature peptide is the preferred approach frequently for the quantification of proteins due to the poor the sensitivity of intact proteins LCMS/MS.

In this study a Q-TOF type instrument was employed in V mode with a mass resolution of 20,000 FWHM. The data was processed to investigate the effect of reducing the mass analysis window from 1 Da, to 100, 40 and 20mDa. The analysis of a 50ng/mL standard extracted protein digest from plasma showed that peak of interest elutes with a retention time of 1.20 minutes ; m/z =616.3821. The use of a 100mDa extraction window significantly improved the specificity of the assay compared with the 1 Da analysis, removing multiple endogenous peaks from the analysis. The 40mDa extraction window the assay showed further improvements in specificity. The blank exhibited no peak with a S/N greater than 3 : 1 eluting at the retention time of 1.2 minutes.

The acquired data was reprocessed using a 20mDa mass window, the results showed that with a 10ng/mL sample an intensity increased by a factor of 1.8 compared to the 40mDa mass window analysis. The 20mDa mass window analysis also showed an improved signal/noise by a factor of 4 over the 40mDa analysis. A typical calibration curve obtained from the 20mDa window analysis had a coefficient of determination (r 2) of 0.994545 with an intercept of 0.00025.

A comparison of the sensitivity of the accurate mass method with a tandem quadrupole methodology showed that the accurate mass method was within one order of magnitude in terms of sensitivity.

Oral Session

Wedensday, 19th September

Room E

09:00 – 11:00

Session 25: New Approaches to Defining the Diversity of Glycans

Chair: Catherine E Costello (Boston University School of Medicine, USA)

S25-0900

09:00 – 09:40

[Keynote Lecture] Development and Application of a Rapid and Sensitive Method for Screening Cellular Models of Congenital Disorders of Glycosylation

Jane Thomas-Oates[1,2,3], Salina Abdul Rahman[1,2,3], Jennifer E Dodd[1,3], Ed Bergstrom[1,2,3], David A Ashford[1,2,4], Jerry Thomas[1,2,4], Daniel Ungar[1,4]

[1]University of York, York, UK, [2]Centre of Excellence in Mass Spectrometry, [3]Department of Chemistry, [4]Department of Biology

Keywords:
congenital disorders of glycosylation (CDG) ; glycan analysis ; protein glycosylation ; permethylation ; filter-aided sample preparation

Novel aspects:
development of simple, fast, sample handling approaches for release and analysis of whole cell glycans ; reducing cell numbers to single culture dishes for medium-throughput glycan screening

Abstract:
Congenital Disorders of Glycosylation (CDG) are a group of rare inherited metabolic diseases caused by defects in protein glycosylation. Most CDG subtypes arise due to a defect in one of the enzymes or transporters in the protein glycosylation pathway. However, there are several subtypes of CDG that arise not by mutation of the enzymes or transporters themselves, but rather from mutations in the components responsible for organising the glycosylation machinery in the Golgi (Wu et al) ; these defects lead to much less predictable patterns of glycosylation and are thus harder to diagnose, to understand, and to treat.

In order to study this class of CDGs, we are developing cellular models of the different patient disorders using appropriate chinese hamster ovary cell mutants (Krieger et al, Ungar et al) . These cellular models allow us to study the effects of such mutations by screening the cellular glycosylation patterns in the laboratory, a particularly important approach given the rarity of diagnosed patients. When potential patients do present, fibroblast samples can be cultured and studied using the same approaches.

We have consequently been developing generic approaches to screening whole cell glycans, with an emphasis on working with realistic cell numbers for medium-throughput screening, and convenient, sensitive, robust, MS-compatible sample handling protocols. We have based our approach on the filter-aided sample preparation procedures described for proteomic analyses (Manza et al, Winiewski et al) and developed these for glycan release, followed by permethylation and MS analysis. The approach relies on solubilising (glyco) proteins from whole cell lysates by heating in SDS, followed by exchange of the SDS for more MS-friendly buffers in a spin filter device. The glycans are then released from the protein mixture above the membrane and the released glycans retrieved from the reaction mixture by centrifugation of the spin filter. Permethylation of the retrieved released glycans makes the samples ready for MS analysis.

The talk will describe our sample handling approaches, illustrated with some of our data from cultured cells.

Acknowledgements
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Oral Session

Wedensday, 19th September

Room E

09:00 – 11:00

Session 25: New Approaches to Defining the Diversity of Glycans

Chair: Catherine E Costello (Boston University School of Medicine, USA)

S25-0940 Studies of Oligosaccharides and Monosaccharides by LEISD MALDI and 2D-CID-ESI Mass Spectrometry Techniques

09:40 – 10:00

shuying Liu[1,2], hongmei Yang[1], Debin Wan[1]

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Keywords:
oligosaccharide, monosaccharide, LEISD MALDI, 2 D-CID-ESI mass spectrometry, isomer

Novel aspects:
This is the first report that carbohydrate isomers were successfully differentiated by LEISD MALDI and 2 D-CID-ESI mass spectrometry.

Abstract:
Studies of Oligosaccharides and Monosaccharides by LEISD MALDI and 2D-CID-ESI Mass Spectrometry Techniques
Hongmei Yang[1], Debin Wan[1] and Shuying Liu[1,2*]
[1]Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 130022, China
[2]Changchun University of Chinese Medicine, 130117, China

Carbohydrates play crucial roles in multiple biological processes including human diseases[1-3]. The complete structural elucidation of carbohydrates is very important to understand their biological functions. Despite the recognized importance, structural analysis of carbohydrates is still challenging due to their enormous structural diversity and micro-heterogeneity.
Here we describe two efficient and practical methods of Mass spectrometry to study the isomer structures of oligosaccharides and monosaccharides.
First, laser enhanced in source decay (LEISD) technique of the MALDI MS allows highly reliable and abundant fragmentation of the neutral oligosaccharides.
MALDI MS technique described to date has proven to be a convenient and rapid method for sequencing purified peptides and proteins. However, for oligosaccharides even the application of the conventional ISD can not produce adequate fragments for the detailed structural elucidation, due to the low internal energy for precursor ions. In this study, we applied an ultrahigh irradiation laser of mJ level to obtain better quality ISD spectra of fragment ions. The LEISD of oligosaccharides demonstrated significantly better signal-to-noise ratio spectra and more structural information obtained. The results showed that the intensities of A-type ions derived from cross-ring cleavage of oligosaccharides allowed the distinction among α (1→4)-, α (1→6)-, β (1→4)-, and β (1→3)-linked isobaric structures according to fragment types and intensities.
We also discussed several parameters (doped metal ions, gas-phase sodium affinity, and proton affinity of the matrices) affecting LEISD of oligosaccharides in detail.
Second, 2D-CID-ESI mass spectrometry was employed to discriminate the isomers of monosaccharides.
Electrospray ionization tandem mass spectrometry (ESI-MS/MS) is another common and well-studied approach for carbohydrate discrimination, according to differences in MS^n fragmentation patterns. Unfortunately, the mass spectra may be too similar to assign isomers based on the product ion ratios.
The collision energy as an additional dimension is added to display the related product-ion information of the isomers in the 2 D-CID mass spectra, which proposed by Han[4]. The CID spectra associate with the internal energy of precursor ions, which mainly depend on collision energy and related reactions. We employed 2 D-CID-ESI-MS in the negative ion mode, and the discrimination of isomers of monosaccharides was achieved completely. With the change of collision energy, the ratios of product ions are detected obviously different among the four isomers, glucose, fructose, mannose, and galactose.

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Oral Session

Wedensday, 19th September

Room E

09:00 – 11:00

Session 25: New Approaches to Defining the Diversity of Glycans

Chair: Catherine E Costello (Boston University School of Medicine, USA)

S25-1000
10:00 – 10:20

A new approach to the rational synthesis of MALDI matrix. Keeping functional groups and its location but changing the stereochemistry

Maria L Salum[2], Lucia M Itovich[2], Hiroshi Nonami[1], Rosa Erra-Balsells[2]

[1]Ehime University, Matsuyama, Japan, [2]University of Buenos Aires, Argentina

Keywords:
isomers, geometric-isomers, trans-cinnamics, cis-cinnamics, carbohydrates.

Novel aspects:
New strategy to prepare MALDI matrix. Improving MALDI MS carbohydrate analysis.

Abstract:
The main idea of the approach followed in the present project for the synthesis of several potential MALDI matrix was based on the fact that each cinnamic acid presents two different isomeric forms : trans-cinnamic and cis-cinnamic. The pair of compounds, are called geometric isomers, in which the nature and position of the functional groups in the benzenic moiety is the same but the spatial relation between de substituent present in the alkenic exocyclic group is different (1,2-anti, trans or *E*; 1,2-gauche, cis or *Z*). As consequence of this different geometric distribution the bulky substituents present at de vicinal 1,2 positions push the molecule to adopt quite different tridimensional structure. Classically for the trans isomer the thermodynamically more stable conformation is almost flat (bencenic and alkene moieties in the same plane) and for the cis isomer bulky substituents at de 1,2 positions brake the molecular planarity and the total volume and tri dimensional structure of the cis isomer is completely different than those of the trans form. As a reality both isomers are different chemical species whose physical, chemical and photochemical properties can be quite different too.

As commercial cinnamic acids (sinapinic, coumaric, ferulic, caffeic, 4-mthoxy-3-hydroxy cinnamic, etc.) are 97-99 % the corresponding trans isomer, we decided to find a proper a technique to prepare the corresponding pure cis forms. Thus, after a one-pot preparation method was developed in our laboratory we started the MALDI experiments. Each pair of trans/cis isomers was inspected in comparative way with regard to its mass spectrum and its performance in protein and carbohydrate MALDI MS analysis.

For our delaight, cis-cinanmic acids showed in general better performance than the trans isomers. In the field of carbohydrates the different behavior is amazing. Particularly cis-sinapinic acid showed to be an excellent matrix for carbohydrate analysis. Thus, neutral sugars (maltoses, fructanes, cyclodextrins, trehalose) showed in positive ion mode, signals with absolute intensity and signal/noise ratio similar or better than those obtained with gentisic acid. Furthermore, in the field of sulfated sugars, cis-cinnamic acids, particularly cis-sinapinic showed better spectra with a drastic diminishing of number and intensity of signals produced by desulfated fragments ; this experiments were conducted comparing the spectra with those got using gentisic acid and nor-harmane as matrix.

For better understanding of the relative performance of the new matrices and its trans isomer computational chemistry (molecular modeling) has been used, after optimization of the geometry, to calculate formation energy, proton affinity, volume, surface area, hydratation energy, log P and dipolar moment.

Oral Session

Wedensday, 19th September

Room E

09:00 – 11:00

Session 25: New Approaches to Defining the Diversity of Glycans

Chair: Catherine E Costello (Boston University School of Medicine, USA)

S25-1020 Automated Identification of Intact Glycopeptides from Complex Samples

10:20 – 10:40

Christopher Becker[1], Marshall Bern[1], Yong Kil[1], Doron Kletter[2], Mudita Singhal[2], Julian Saba[3], David Horn[3], Sergei Snovida[4], Rosa Viner[3]

[1]Protein Metrics Inc., [2]Palo Alto Research Center, Palo Alto, CA, USA, [3]Thermo Fisher Scientific, San Jose, CA, USA, [4]Institute of Biological Chemistry, Academia Sinica, Taipei, TAIWAN

Keywords:
glycopeptide identification, glycoproteins, glycosylation

Novel aspects:
Large-scale intact glycopeptide identification by integrated HCD and ETD analysis and database searching

Abstract:

Introduction

About half of all human proteins are believed to be glycosylated, and glycosylation plays a major role in both normal cell signaling and disease processes. Understanding glycosylation on an "omics " scale, as well as discovery of glycoprotein disease biomarkers, however, has been limited by the analytical challenge of identifying glycopeptides in complex samples such as blood plasma or cellular extract. Here we present a new database search engine named Byonic that allows large-scale glycoprotein identification, including glycans. Byonic was applied to product-dependent Orbitrap data that uses HCD-generated MS/MS fragments to target glycosylated peptides for ETD analysis in the following scan.

Methods

Human blood plasma and cellular protein samples were digested by trypsin and enriched for glycopeptides by strong anion exchange, and then labeled with Tandem Mass Tags (TMT 0) to improve ionization efficiency and increase precursor charge state distribution, yielding superior ETD fragmentation. Product-dependent ETD acquisition uses characteristic glycoside (138.055 Da) and oxonium ion peaks (204.087, 366.139 Da) in high-accuracy HCD Orbitrap scans to trigger ETD scans of glycopeptides. Byonic data analysis uses expert-systems, for both fragmentation modeling and O- and N-linked glycan table generation, to make identifications from search spaces that can involve ~ 1000 glycan forms and > 4000 proteins, with > 10,000 potential N-glycosylation sites, and roughly a million potential O-glycosylation sites.

Preliminary Results

Glycopeptide identification is limited by relatively poor fragmentation and very large search spaces, even with the advances of TMT labeling and product-dependent ETD data acquisition. The size of the search space is dependent upon the product of the number of peptides with potential glycosylation sites, the number of glycan masses, and the number of combinations of other modifications (sodiation, potassiation, methionine oxidation, etc.) . With Modification Fine Control capability, Byonic allows the user to customize the search with separately settable limits on each modification type, capped by a limit on the total number of modifications. This avoids the all-to-familiar combinatorial explosion of other search engines while providing great flexibility and a wide range of types of modifications allowed. We used a table of the ~600 most plausible mammalian N-glycan masses, as determined by Cartoonist, expert-system software for glycan profiling. From plasma proteins, of 2540 ETD scans almost sure to contain glycopeptides, Byonic found identifications for 700 scans with accuracy > 90%, as judged by manual curation and prior knowledge (Uniprot KB) . Computation time was less than a few minutes on a desktop PC. Investigation of cellular samples will also be reported. We compare independent HCD / ETD analysis with integrated HCD / ETD analysis, in which peaks in the HCD spectrum focus attention on glycopeptides containing, for example, fucosylated or sialylated glycans. We also show the effect of limiting the search space along the three dimensions of protein, glycan, and other-modification complexity.

Oral Session

Wedensday, 19th September

Room E

09:00 – 11:00

Session 25: New Approaches to Defining the Diversity of Glycans

Chair: Catherine E Costello (Boston University School of Medicine, USA)

S25-1040

10:40 – 11:00

Ion mobility mass spectrometry of IgG Fc glycopeptides from different subclasses

Michiko Tajiri[1], Yayoi Hongo[2], Takemichi Nakamura[2], Kenji Hirose[3], Yoshinao Wada[1]

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Keywords:
ion mobility MS, glycoproteomics, glycopeptide, glycan-peptide interaction, collision cross-section

Novel aspects:
Ion mobility MS of glycopeptides suggests an interaction between N-glycan and peptide backbone sequence to specify different IgG subclass functions.

Abstract:

Introduction

IgG molecules are glycosylated with a complex biantennary-type oligosaccharide at Asn-297 of the CH2 domain within the Fc region. These glycans are essential for Fc effector functions of IgG such as complement activation and Fcγ receptor-mediated activities. These functions have been studied mainly for IgG1 among four subclasses whose structures are different each other in the residues Y (Tyr) or F (Phe) at 296 and 300. Considering the differential affinities of IgG subclasses to Fcγ receptors, we hypothesized an interaction between glycan and amino acid residues near the glycosylation site, and analyzed glycopeptides containing the region by ion mobility MS to address the issue.

Methods

The tryptic glycopeptide from IgG Fc region were enriched by a hydrophilic affinity method using Sepharose CL-4B (Wada *et al.*, Anal Chem 76：6560, 2004). Truncation of oligosaccharides was carried out by specific glycosidic enzymes, and the glycopeptides from different IgG subclasses were separated each other by reversed phase chromatography. The glycopeptides were dissolved in 20 % methanol containing 0.1 % formic acid, and the sample solution was loaded in a nanospray tip for ion mobility MS using a Waters SYNAPT G2 system. The drift time of doubly protonated molecules of glycopeptides was measured. For CID, the ions were activated in the Transfer T-wave collision cell. For calibration of collision cross section, tryptic peptides of BSA, bovine apo-transferrin, human transferrin and horse myoglobin were employed according to a report by Valentine *et al.* (JASMS 10：1188, 1999).

Results

The amino acid sequences of tryptic glycopeptides from the IgG1 and IgG2 Fc regions are EEQYNSTYR (exact mass 1188.5；296Y/300Y) and EEQFNSTFR (1156.5；296F/300F), respectively, and the N-glycans are attached to Asn297 in the middle of the sequence. The drift time of doubly protonated molecules of the synthetic peptides without glycans was normally dependent on the molecular mass of these peptides. The protonated molecular ions of IgG1 glycopeptide bearing mature glycans displayed a smaller collision cross section relative to IgG2, despite the former is larger in the molecular mass. This feature of conformational change was found in the glycopeptide bearing a single GlcNAc attached to Asn297, suggesting an interaction between the innermost GlcNAc and peptide backbone sequence. Analysis of IgG3 (296Y/300F) and IgG4 (296F/300Y) glycopeptides suggested that both 296 and 300 were involved in the interaction. More specifically, regarding the glycopeptide bearing core trisaccharide ($GlcNAc_2Man$), IgG1 or IgG4 displayed smaller collision cross section than IgG2 or IgG3 glycopeptides, suggesting a contribution of Tyr300. Finally, an energy-resolved CID experiment suggested the compact conformation of IgG1 glycopeptide ions compared with IgG2 ions, apparently consistent with the results described above.

Conclusion

The contribution of innermost GlcNAc of N-glycans to the entire acceleration of folding and native state stabilization of glycoproteins has been reported by Hanson *et al.* (PNAS 106：3131, 2009). Our findings suggested that the aromatic residues at 296 and 300 of IgG Fc region are involved in the GlcNAc-peptide interaction, thereby making differences in the biological properties of four IgG subclasses.

Oral Session

Wedensday, 19th September

Main Hall

15:00 – 17:00

Session 26: Lipidomics : Recent New Techniques and Applications

Chair: Stephen J Blanksby (University of Wollongong, Australia)

S26-1500
15:00 – 15:40

[Keynote Lecture] High-Resolution 'Shotgun' Mass Spectrometry and Chemical Labeling for Comprehensive and Quantitative Lipidome Profiling of Disease

Cassie J Fhaner[1], Hong Ji[2], Richard J Simpson[2], Gavin E Reid[1]

[1]Michigan State University, USA, [2]Latrobe University, Australia

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Keywords:
'fixed-charge derivatization ; colon adenocarcinoma ; lipidomics

Novel aspects:
Chemical derivatization enables enhanced lipidome using ultra-high resolution 'shotgun' mass spectrometry

Abstract:
A large number of studies have demonstrated that disruption of lipid metabolism or signaling can play a key role in the onset and progression of human diseases, including cancer and diabetes. Thus, comparative analysis of changes in individual lipids or lipid profiles (i.e., the lipidome) between normal and diseased cells, tissues or accessible bodily fluids, may enable the identification and characterization of lipids that can serve as effective biomarkers of the disease. In this presentation, we will describe the development and application of a straightforward and high throughout analysis strategy consisting of ultra high-resolution ' shotgun' mass spectrometry (MS) coupled with functional group specific chemical modification and 'targeted' MS/MS for the identification, characterization and quantification of >600 individual glycerolipid, glycerophospholipid, sphingolipid and sterol lipid species from whole cell or tissue samples, across more than 4 orders of magnitude dynamic range of abundance, and with minimal need for sample handling or lipid extraction prior to analysis.

Oral Session

Wedensday, 19th September

Main Hall

15:00 – 17:00

Session 26: Lipidomics : Recent New Techniques and Applications

Chair: Stephen J Blanksby (University of Wollongong, Australia)

S26-1540
15:40 – 16:00

Tandem MS Methods For Assignment Of Double Bonds And Chain Branching In Fatty Acid Methyl Esters

Tom Brenna, Rinat Ran-Ressler, Peter Lawrence
Cornell University, Ithaca, New York, USA

Keywords:
lipidomics, FAME, BCFAME, chemical ionization

Novel aspects:
Lipidomic methods have traditionally ignored isomeric chains ; this paper highlights emerging methods for determining structure via MS.

Abstract:
The position of double bonds and chain branching in fatty radyl chains determines their chemical and biological properties. Conventional lipidomics methods provide carbon number and double bond number of fatty radyl chains in their native lipid class, but do not provide information on isomeric structure. The most common analytical form of fatty radyl groups in a wide range of biomedical and biochemical studies is as fatty acid methylesters (FAME) because of the very high resolving power of capillary gas chromatography. Neither electron ionization nor conventional chemical ionization provide ions diagnostic of double bond position or chain branching in FAME. The usual means to evaluate these isomers is by specialized derivatization with charge localizing groups (e.g. DMOX, picolinate) . Apart from the need for special chemistry, these techniques alter retention times, can induce double bond migration, and reduce sensitivity, all of which present difficulties. For more than a decade we have developed covalent-adduct chemical ionization tandem mass spectrometry for assignment of double bond position in monoene, honoallylic polyene, conjugated diene, and several other classes of conjugated polyene FAME. Under CI conditions, acetonitrile (CH_3CN, MW=41) self reacts to form a reagent ion $[CH_2{=}C{=}N{=}CH_2]^+$ (*m/z* 54) which undergoes an ion-molecule reaction with double bonds to form cyclic structures. Collisional activation yields a small number of diagnostic ions that reveal double bond position. Fragmentation is systematic and depends on the double bond configuration, either vinylic or allylic to the site of the original double bond, and occurring with transfer of a H either to or from the neutral. The method is particular amenable to quantitative analysis since a small number of strong ions are formed. We extended these results to atmospheric pressure ionization of triacylglyerols with mono and diene fatty acyl groups. More recent work has focused on branching in saturated monomethyl branched chain FAME (BCFAME) . BCFAME are prominent in cow and other ruminant milks, beef and other ruminent meat, bacteria, and in certain human substances generally related to skin secretions. EI spectra provide fragments that enable assignment of branching position in many but not all cases. Zirroli and Murphy (JASMS, 1993) showed that the EI-derived molecular ions of a few BCFAME are rearrangement products that, upon further collisional dissociation, produce highly specific and strong fragment ions. We recently tested this method for a broad range of BCFAME from C12 to C31. Bond breaking was on the carboxyl side of the branch point for *iso* BCFAME yielding an isopropyl neutral loss. For most other BCFAME, bonds on both sides of the branch point break yielding two high intensity fragments. In all cases the expected diagnostic ions were observed, enabling unambiguous assignment of branch structure. The method was applied to vernix caseosa, the white, waxy substance found on the skin of human newborns, and for BCFAME found in lanolin. These very convenient and rapid methods improve the confidence with which the very diverse set of isomers found in FAME can be assigned.

Oral Session

Wedensday, 19th September

Main Hall

15:00 – 17:00

Session 26: Lipidomics : Recent New Techniques and Applications

Chair: Stephen J Blanksby (University of Wollongong, Australia)

S26-1600
16:00 – 16:20

The Missing Link between Neurodegeneration and Sphingolipid Metabolism: Integration of Lipidomics and Proteomics

Sarita Hebbar, Mayura Nakade, Dominik Schwudke

National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India

Keywords:
Lipidomics, Label-Free Quantitative Proteomics, Sample Preparation, Drosophila melanogaster, Sphingolipids

Novel aspects:
We present an analytical strategy enabling us to perform simultaneously quantitative lipidomics and proteomics experiments from small tissue samples.

Abstract:
In recent years the importance of lipid metabolism in maintaining the integrity of the nervous system was established in a number of studies [reviewed in (1)] . Perturbations of lipid biosynthetic pathways due to stress and pathological processes are of complex and quantitative in nature. *D.melanogaster* is the ideal model for this study because of the wealth of genetic tools available to modulate lipid metabolism and the simplicity of its cultivation. The aim of this study was to develop efficient sample preparation strategies for integrating proteomics and lipidomics approaches for studying *Drosophila* lipid biology.
We developed a workflow which enabled us to routinely perform lipidomics and proteomics measurements from only 5 dissected adult brains. Dissected adult brains were processed with the MTBE lipid extraction (2) . The upper organic layer was utilized for shotgun lipidomics and LC-MS/MS (3,4) . Lipidomics analysis was performed on a hybrid LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a robotic nanoflow ion source TriVersa (Advion BioSciences Ltd, Ithaca NY) . Liquid chromatography was performed on a 1200 micro-LC-system (Agilent Technologies, Waldbronn, Germany) with a split flow setup enabling nano-ESI-MS. Mass spectrometric data interpretation was performed with proprietary software LipidXplorer (5) and Xcalibur (Thermo Fisher Scientific, Bremen, Germany) . The lower aqueous layer was processed for total protein determination and proteomics starting with 1D SDS-PAGE analysis. Total protein amounts were determined by image analysis of coomassie stained gels using Image J. Gel lanes were sliced into 11 pieces which were processed for In-gel digestion. The extracted peptides were spiked with BSA peptides and analyzed by Agilent 1200 NanoLC system and a LTQ-Orbitrap Discovery (Thermo Fisher Scientific, Bremen, Germany) coupled with an Advion Nanomate Triversa. MASCOT database searches and label-free quantitation were performed using Proteome Discoverer 1.3 (Thermo Fisher Scientific, Bremen, Germany) . In the course of such experiment we can access quantitative information of approximately 800 proteins, 200 lipids, and determine the total protein content of a sample. For the analysis of such small samples amounts we apply the abundance of housekeeping and structural protein as reference for lipid quantities. Furthermore, we can evaluate the efficiency of genetic manipulation of biosynthetic genes on the protein level as exemplified by Ceramidase (Dmel ¥CDase) over-expression.
Currently we apply this methodology to analyze the influence of lipid metabolic perturbation in spinster (Dmel ¥spin) mutants (6) , an established model for Lysosomal Storage Diseases (LSD) . We could show that the abundance levels of ceramides are preceding the manifestation of neurodegenerative pathology as characterized by electron microscopy and behavioral assays. Finally, we show that membrane lipid classes like PE, PC and CerPE start to accumulate in the brain in conjunction with the appearance of abnormal structures like membrane whorls and lipofuscin. We will now manipulate sphingolipid metabolic pathways in the brain using the UAS-Gal4 system in the background of the Spinster mutation.

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Oral Session

Wedensday, 19th September

Main Hall

15:00 – 17:00

Session 26: Lipidomics : Recent New Techniques and Applications

Chair: Stephen J Blanksby (University of Wollongong, Australia)

S26-1620

16:20 – 16:40

What is a complete lipidome? A snipers approach to shotgun analysis of the human lens.

Jane M Deeley, Jessica R Hughes, Shane R Ellis, Jo Ann Seng, Roger J Truscott, Stephen J Blanksby, Todd W Mitchell

University of Wollongong, Wollongong, Australia

Keywords:
Lipidomics, ozone-induced dissociation, TLC-DESI, ambient ozonolysis, MS imaging

Novel aspects:
We have developed new mass spectrometry based techniques for lipid characterization and used them to identify novel lipids in the human lens.

Abstract:
The lens is an essential component of vision, allowing light impinging upon the eye to be focused onto the retina. Presbyopia and cataract are age-related disorders of the lens and are the two most common causes of visual impairment and blindness worldwide. Considerable knowledge has been attained regarding the biochemistry of lens proteins and their possible role in the development of these diseases, yet our knowledge of lens lipids is limited in comparison. To address this disparity we have utilized numerous modern mass spectrometry techniques, e.g. shotgun lipidomics, MALDI imaging, DESI imaging, liquid extraction surface analysis (LESA) and TLC-DESI and novel techniques, e.g. ozone-induced dissociation (OzID) and TLC-ambient ozonolysis to investigate the lipid composition of the human lens and how it is altered as we age. Through the use of OzID we have been able to identify that most phospholipid classes in the lens are dominated by alkyl ethers and that numerous double bond isomers are found in both the ether and ester-linked acyl chains. For example, the most abundant glycerophospholipid ion observed during MS analysis of human lens lipids extracts was found to be a mixture of up to four double bond isomers, i.e. PE (11*Z*-18 : 1e/9*Z*-18 : 1), PE (9*Z*-18 : 1e/9*Z*-18 : 1), PE (9*Z*-18 : 1e/11*Z*-18 : 1) and PE (11Z-18 : 1e/11Z-18 : 1). OzID and TLC-ambient ozonolysis-DESI have also uncovered the presence of several molecular lipids that had not previously been identified, e.g. PS (13*Z*-18 : 1e/9*Z*-18 : 1 and SM (d18 : 0/19*Z*-24 : 1). Through the use of TLC-DESI we have also obtained the first identification of molecular sulfatides, dihydrosulfatides, lactosylceramide sulfates and dihydrolactosylceramide sulfates within the human lens. Based on this information we have developed specific precursor ion and neutral scans for the detection and quantification of these lipids using a shotgun lipidomics approach or directly from lens tissue using LESA. Finally, we have also been able to observe large changes in both the concentration and distribution of molecular lipids in the lens with age utilizing shotgun lipidomics in combination with both MALDI and DESI imaging.
From the example of the human lens it is clear that there is no single mass spectrometry platform capable of comprehensively analyzing a complete lipidome. Through the use of various techniques however, we are edging towards such an understanding in the human lens. In this talk I will discuss some of the techniques we have developed and how we have applied them to the characterization and measurement of molecular lipids in the human lens.

Oral Session

Wedensday, 19th September

Main Hall

15:00 – 17:00

Session 26: Lipidomics : Recent New Techniques and Applications

Chair: Stephen J Blanksby (University of Wollongong, Australia)

S26-1640

16:40 – 17:00

Toward Total Structural Analysis of Complex Lipids: Electrospray Ionization Multiple-stage Linear ion-trap with High-resolution Mass spectrometric Analysis of Intact Ions

Fong-Fu Hsu

Washington University School of Medicine in St. Louis

Keywords:
Complex lipids ; Double bond localization ; Multiple-stage mass spectrometry ; glycerolipids

Novel aspects:
This report presents linear Ion-trap MSn with high-resolution mass spectrometry for complete structural characterization of complex lipids that would be difficult to define using traditional approaches

Abstract:
Recent development in mass spectrometric techniques has advanced lipid research to a new level. The mass spectrometric approaches facilitate characterization of complex lipid structures, including features such as the identity of fatty acid substituents, their *sn*-position on the glycerol backbone, and the positions of carbon-carbon double bonds along the acid chain of the glycerolipids. Among the various approaches including high-energy collision induced dissociation, covalent adduct chemical ionization, and more recently, ozone-induced dissociation and low-energy collision induced dissociation in line with multiple-stage (MS^n) linear ion-trap (LIT) mass spectrometry, the latest two methods are the most comprehensive and allow a near complete identification of the lipid structure. In this presentation, I will describe LIT MS^n with high-resolution mass spectrometry to achieve structural determination of complex lipid isolated from biological specimen by applying (1) MS^n on the $[M - H]^-$ ions for definition of the lipid class and identification of the fatty acid substituents, as well as determination of their regio-specificities, (2) MS^n on the corresponding $[M + Li]^+$ or $[M - H+ 2 Li]^+$ ions for confirmation of the structure assignment and more importantly, locating the double bond (s) , cyclic chain and branch chain positions of the fatty acid substituents. The fragmentation pathways for formation of the fragment ions arising from rearrangement processes and leading to structural assignments will be proposed. Examples including differentiation among isomers and revelation of isomeric structures in a mixture using high resolution and MS^n mass spectrometry will be given and the prospects of this analytical method in the lipid study in the future, in particular, in the structure determination of complicate microbial lipids will be discussed.

Oral Session

Wedensday, 19th September

Room A

15:00 – 17:00

Session 27: Progress in Microbiology

Chair: Catherine Fenselau (University of Maryland, USA)

S27-1500

15:00 – 15:40

[Keynote Lecture] Translating metabolic phenotyping and systems medicine into clinical Practise: Understanding gut microbial-host Interactions in personal and public healthcare problems

Jeremy K Nicholson

Imperial College London

Keywords:
Microbiome Metabolism Healthcare Stratified Medicine

Novel aspects:
Spectroscopic modelling of complex micro biome-host metabolic interactions to create new healthcare and drug targeting opportunities

Abstract:
The composition, activities and function of the gut microbiome in health and disease is of currently of great interest in basic human biology and medical research. Multiple and diverse discoveries on the close associations between microbiome variation and the etiopathogenesis of many common non-infectious diseases has lead to new research initiatives designed to answer key microbiome questions about "Who is there ; what are they doing ; how did they get there ; what are their functions ; what happens if things go wrong and how do we manipulate them for human health benefits? " To answer these questions on the functional properties of microbiomes requires systems approaches that go well beyond metagenomic mapping as it is the response of the host to microbiological variance that is of critical importance in human health and for the informed use of microbiome data in the development of new therapeutic approaches. This involves deep metabolic phenotyping via spectroscopic methods. The extensive cross-talk between the host and the gut microbiome at the metabolic control and signalling level that is modulated in exquisitely complex ways by genes and environment and link to disease risk factors (1,2) . These symbiotic supraorganismal interactions greatly increase the degrees of freedom of the metabolic system that poses a significant challenges to fundamental notions on the nature of the human diseased state, the etiopathogenesis of common disease and current analytical modelling requirements that are required for future developments in personalised healthcare and "precision medicine " (3,4) . The principal analytical challenges involve integrative analysis of biofluid metabolic signatures via mass spectrometric and NMR spectroscopic methods in relation to to microbiome variation and the extraction of biomarker patterns and models that can be used to understand human disease processes and/or their treatments. Here we will explore the systems analytical approaches to understanding the functional aspects of microbiome activity and describe possible discovery roadmaps to elucidate the many roles of the microbiome in human health and disease.

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S27-1540
15:40 – 16:00

Distribution and identification of molecular interactions between tomato roots and bacterial biofilms

Delphine Debois[1], Emmanuel Jourdan[2], Nicolas Smargiasso[1], Marc Ongena[3], Philippe Thonart[2], Edwin De Pauw[1]

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Keywords:
MALDI-Imaging, Plant-bacteria interaction, lipopeptides, biofilm

Novel aspects:
MALDI-Imaging allowed mapping tomato roots-biofilm interactions. Different lipopeptides families were shown to be produced according to the age of the root-biofilm system. Finally, a putative new lipopeptide family was detected.

Abstract:
Some non pathogenic microorganisms evolving in the root micro-environment can trigger a positive effect on plant, increasing host defense against disease or/and directly inhibiting growth of pathogen in soil (1). To initiate both phenomena leading to biocontrol activity, microorganisms use plant exudates to grow on roots and to produce *in-situ* active compounds. In Bacilli, cyclic lipopeptides of the surfactin, iturin and fengycin families represent important antibiotics involved in biocontrol (2). Recent studies in microbiology allowed a better understanding of plant microorganism interactions but few has been done at the molecular level. In this study, MALDI-MS imaging has been used to study the nature of the secreted lipopeptide molecules, their relative quantity and their distribution in the root's environment.

Disinfected tomato seeds were first germinated at 28°C in sterile conditions for germination. Seedlings were then placed in Petri dish on ITO glass slide recovered with a thin layer of plant nutritive solution (Hoagland) containing 1,75% of agar and treated with freshly-grown cells of *Bacillus amyloliquefaciens* S499. Petri dishes were finally incubated vertically in phytotron at 28°C with a 16h photoperiod. Different root age / time of incubation were studied : 13 / 3 ; 13 / 7 ; 21 / 14 and 39 / 32. Control tomato root (without bacterial treatment) of the same ages were also analyzed (13 / 0 ; 21 / 0 and 42 / 0. For MALDI imaging experiments, the ITO slide was removed from the agar and dried in a dessiccator under vacuum. The matrix solution (α-cyano-hydroxycinnamic acid, 5 mg/mL in ACN/0.2 % TFA 70/30) was applied with an ImagePrep automated sprayer (Bruker Daltonics). An UltraFlex II TOF/TOF and a Solarix FT-ICR mass spectrometers were used to record molecular cartographies.

The average mass spectra recorded around the tomato root (2-3 mm on both sides of the root) showed that lipopeptides were major compounds detected on the agar. The relative intensity of lipopeptides families varied with respect to the age of the root/biofilm system. In the 13/3 system, 3 homologues of surfactins were essentially detected (C13, C14 and C15), with very few iturins and fengycins. Their localizations were identical, whatever the considered homologue. Then the production of iturin and fengycin families increases in older systems (13/7 and 21/14) and a novel homologue of surfactin is detected (C12). Some variations in localization within families may be observed (around the root or at the close vicinity of it in function of the considered homologue or alkali adduct). Then for the oldest system we studied, iturins and fengycins are not detected anymore and the localization of surfactins is less precise. In the 39/32 system, we also detected unknown compounds at 986.6, 1000.6, 1014.7 and 1028.7 m/z. The mass range of these compounds allied to the mass difference between two consecutive ion peaks let us think that these unknown compounds could be a new lipopeptide family. Investigations are in progress to identify these new secondary metabolites of *Bacillus amyloliquefaciens*.

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Oral Session

Wedensday, 19th September

Room A

15:00 – 17:00

Session 27: Progress in Microbiology

Chair: Catherine Fenselau (University of Maryland, USA)

S27-1600
16:00 – 16:20

Identification of Protein Biomarkers as Diagnostic Targets for Invasive Aspergillosis using Discovery and Targeted Proteomics

Chengsi Huang[1,3], Jason McCarthy[2], Marta Feldmesser[2], Vicki H Wysocki[1,3]

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Keywords:
Aspergillus, Discovery/ Targeted Proteomics, Plasma

Novel aspects:
The ability to detect Aspergillus fungal proteins in host plasma in pulmonary diseases ; identification of same Aspergillus protein in both mouse and human plasma.

Abstract:
Aspergillus fumigatus is a ubiquitous fungus. While an average individual's healthy immune system will effectively clear out the conidia inhaled, immunocompromised patients may develop invasive aspergillosis (IA) , a potentially fatal disease. Current diagnostic tests can be time-consuming and/or invasive, and have variable accuracy. Consequently, patients with IA often receive treatment too late. In addition, *A. fumigatus* infection can lead to other diseases such as aspergilloma, or allergic bronchopulmonary aspergilloma. Hence, development of a fast, sensitive, and less invasive diagnostic technique is critical to being able to correctly and confidently identify IA in patients. The goal of the present study is to identify *A. fumigatus* proteins that are specifically present during IA. The identified target proteins will help develop future diagnostic tools.

In this study, mouse is used as the model system. CBA/J mice were divided into three different groups : naïve control, asthma-model, and IA-model. Lung homogenate, BAL fluid, plasma, and serum were collected from all mice. A bottom-up proteomics approach was utilized for analysis. Due to the complexity of the sample, several purification treatments were implemented and optimized in order to provide the greatest number of protein identifications. Plasma samples were depleted via mouse spin columns (Seppro® Mouse) . The depleted then digested samples were analyzed via LC-MS/MS on the Thermo Scientific LTQ Velos Orbitrap via either general discovery methods or targeted methods. Results were searched against a database composed of *Aspergillus fumigatus*, *Mus musculus*, and common contaminants using various algorithms for protein identification.

The mouse plasma samples yielded identification of *A. fumigatus* proteins. By comparing the three models, differential expression of A. fumigatus proteins was found. We found several *Aspergillus* proteins with confident two peptide hits that were exclusive to the IA sample. These proteins, however, exhibit shared homology to other organisms, and are hence are not specific enough to serve as diagnostic targets. Based on multiple spectral count single peptide hits, we also found numerous proteins that are exclusive to either the IA sample or the asthma sample. Out of these proteins, through multiple sequence alignment, we generated a list of approximately thirty possible candidates that are *fumigatus* only, *pan-aspergillus*, and/or pan-fungal. The presence of these proteins is confirmed via targeted analysis on the Orbitrap in individual mouse samples. Meanwhile, we also observed similar host protein patterns and the same *Aspergillus* protein in a patient sample. This gives confidence in using mouse models for human diagnostic study development.

Oral Session

Wedensday, 19th September

Room A

15:00 – 17:00

Session 27: Progress in Microbiology

Chair: Catherine Fenselau (University of Maryland, USA)

S27-1620

16:20 – 16:40

Microbial genome mining for novel bioactive natural products with high resolution LC-MS/MS

Lijiang Song[1], JuanPa Gomez-Escribano[2], Luisa Laureti[3], Eshwar Mahenthiralingam[4], Bertrand Agile[3], Meryvn Bibb[2], Gregory Challis[1]

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Keywords:
genome mining, microbial drug discovery

Novel aspects:
Genome mining as a novel efficient approach for antimicrobial natural product discovery with mordern analytical tools combined with molecular microbiology and bioinformatic techniques.

Abstract:
Antimicrobial resistance is a growing health problem worldwide and there is an urgent requirement for new antibiotics to deal with life-threatening infection, such as those caused by MRSA and VRSA. Meanwhile, the pace of discovery of novel antibiotics has been slowed significantly by the frequent re-isolation of known compounds with the traditional bioactivity-based screening approach, which led to the notion that most natural products had already been discovered, and prompted many pharmaceutical and agrochemical companies to terminate their natural product discovery programs. However, with many microbial genome sequences becoming available, a successful new approach, genome mining, has been developed in recently years. This new approach benefits in particular from modern chromatography, mass spectrometry and NMR spectroscopy, combined with modern molecular biology and bioinformatics tools. Many novel bioactive natural products have been discovered in an efficient manner by this approach not only from the well-known antibiotic producer, the gram positive actinomycetes, great potential has also been realized in gram negative bacterial, such as *Burkholderia*.
Large number of annotated bacterial genome sequences are completed and deposited in varies database, many of them are freely available in the public domain, target gene clusters, in particular, PKS and NRPS, (mainly encoding polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs)) were located and mutants with biosynthetic genes deleted or regulator genes activated or overexpressed were prepared. Structural information of potential products of such gene clusters can often be predicted by sequence analysis. Comparative metabolite profiling using LC-MS/MS and targeting predicted structural properties of metabolic products led to their identification.
This talk will cover the identification, isolation and structure elucidation of coelimycin P, an unusual yellow-pigmented metabolic product of the *cpk* cryptic polyketide biosynthetic gene cluster of *Streptomyces coelicolor* M145, using a genetic engineering strategy designed to increase metabolic flux through the biosynthetic pathway. The discovery of a family of novel 51-membered macrolide antibiotics, the stambomycins, as the metabolic products of the *Streptomyces ambofaciences* by activation of a silent type I PKS gene cluster will be discussed. The identification of enacyloxin IIa as the product of the cryptic PKS gene cluster and insight into enacyloxin biosynthesis provided the basis for potential genetic engineering to generate more analogues. This new genome mining approach, which relies on modern LC/MS/NMR instrumentation, has been shown to be a highly successful approach for the discovery of novel bioactive natural products.

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Oral Session

Wedensday, 19th September

Room A

15:00 – 17:00

Session 27: Progress in Microbiology

Chair: Catherine Fenselau (University of Maryland, USA)

S27-1640

16:40 – 17:00

Novel accurate bacterial identification by MALDI-TOFMS based on ribosomal protein coding in S10-spc-alpha Operon at Stain level

HIROTO TAMURA[2], YUDAI HOTTA[2,3], HIROAKI SATO[4], KEISUKE SHIMA[1], AKIFUMI HOSODA[2], NORIYUKI OJIMA[1]

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Keywords:
MALDI-TOFMS, bacterial identification, bioinformatics, ribosomal protein, database search

Novel aspects:
Novel Accurate MALDI-TOFMS bacterial identification method at strain level, named S10-GERMS method, using ribosomal proteins coded in S10-spc-alphaoperon as biomarkers, which are theoretically calculated and validated.

Abstract:
Matrix-assisted laser desorption ionization-time-of flight mass spectrometer (MALDI-TOFMS) is one of the most widely used mass-based approaches for bacterial identification and classification because of simple sample preparation and extremely rapid analysis within a few minutes without any substantial costs for consumables. Two approaches are utilized for MALDI-TOFMS bacterial identification. First approach is the pattern matching between the experimental MS spectrum of unknown bacteria and MS spectra of known bacteria species in database. This method can realize stable bacterial identification at species level independent on culture conditions and operator's skill, because database includes only *m/z* of the peak, which is stably detected in mass spectrum of bacteria at any conditions. Some bacteria identification systems on the basis of pattern matching are commercialized and are rapidly expanding to clinical, food safety sterility testing and so on. However, pattern matching methodology cannot assure to identify bacteria at strain level, because infrequently detecting strain specific ion peaks are almost missed in database.
In biomarker finding as second approaches, *m/z* of species or strain specific ion peaks are theoretically calculated and corresponding peaks to theoretical peaks are found in experimental MS spectra for bacterial identification. This method has high potential to identify bacteria at strain level, because strain specific peaks are known and validated before measurement. However, the bacterial identification method based on the bioinformatics approach has several caveats ; (1) the being identified or a closely related strain must have been genome-sequenced ; (2) the accuracy of the genomic information is critical ; (3) because of post-translational modification, there can be a significant difference between the theoretical mass based on a genomic database and the observed mass of the protein by MALDI-TOFMS. To avoid uncertain biomarker peaks, we are proposing the bacterial identification method at the strain level using ribosomal subunit proteins as biomarkers for the rapid identification of bacteria. Because ribosomal subunit proteins have fewer post-translational modifications except for N-terminal methionine loss, it is easy to calculate theoretical biomarker masses of the genomic-sequenced strain. Finally, the ribosomal subunit proteins coded in the S10-spc-alphaoperon, which encodes half of the ribosomal subunit protein and is highly conserved in eubacterial genomes, were selected as reliable biomarkers for bacterial identification at strain level.
We have already reported that our proposal method could successfully distinguish the difference at strain level because of mass difference of 2 ribosomal subunit proteins, despite the difference of only 2 bases in the 16S rRNA gene between two strains of genus Bacillus. In this presentation, we reported other application examples of bacterial identification at strain level and propose newly accurate, robust and easy bacterial identification method by combining pattern matching and biomarker method.

Oral Session

Wedensday, 19th September

Room B-1

15:00 – 17:00

Session 28: IR Spectroscopy of Gas-phase Ions

Chair: Dietmar Kuck (Bielefeld University, Germany)

S28-1500 [Keynote Lecture] IR Photodissociation Spectroscopy: A new dimension to Tandem Mass Spectrometry

15:00 – 15:40

Philippe Maitre

Paris Sud University / CNRS - Orsay - FRANCE

Keywords:
Specific Ion Activation ; Ion Spectroscopy ; IRMPD ; Peptide Fragmentation ; Gas-Phase Ion Structure

Novel aspects:
The coupling of tunable infrared lasers with commercial tandem mass spectrometer offers a specific activation method for deriving structural information and thereby provides a new dimension to mass spectrometry.

Abstract:
Recent coupling of tunable and highly intense pulsed IR laser sources with commercial tandem mass spectrometer offers a new dimension to mass spectrometry. Free-Electron-Laser (FEL) facilities such as FELIX (Nieuwegein, Netherlands) and CLIO (Orsay, France) have recently been shown to be particularly well suited IR light sources for performing IR spectroscopy of mass-selected molecular ions. FELs are particularly interesting since they produce tunable mid-IR radiation in a broad wavenumber range covering the so-called molecular fingerprint region (typically 600-2000cm^{-1}) , and their high intensity makes them very suitable for IRMPD (IR Multiple Photon Dissociation) spectroscopy of gas phase molecular ions.
At CLIO, experiments are performed using two commercial instruments : a quadrupole ion-trap (QIT) or a 7 teslas Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer coupled with the IR FEL. In addition, IR spectra can be recorded in the NH and OH stretching region using an optical parametric oscillator/amplifier (OPO/OPA) laser system. In this case, in particular, an auxiliary CO_2 laser can be used for improving the sensitivity of the spectroscopy.
Understanding the structures, energetic, and decomposition pathways of the gas phase peptide fragments is central to the widely used "bottom-up " approach for proteome sequencing based on mass spectrometry. We will focus our attention on the structure of N-terminal CID fragments (a_n^+or b_n^+) of peptides. We will see that the 1600-2000 cm^{-1} spectral range is the most diagnostically useful part of the IR spectrum, especially for distinguishing oxazolone from other isomers of b_n^+ ions. IR spectra of larger b_n^+, and also a_n^+, ions will also be presented providing evidence of the formation of a macro-cyclic structure. The isomerisation of peptide fragments will be discussed in light of very recent results, and we will see that IR signatures of rearranged peptide fragments can also be found in the NH and OH stretching region.

Oral Session

Wedensday, 19th September

Room B-1

15:00 – 17:00

Session 28: IR Spectroscopy of Gas-phase Ions

Chair: Dietmar Kuck (Bielefeld University, Germany)

S28-1540

15:40 – 16:00

Intracavity infrared multiple photon dissociation spectroscopy of transition metal carbene cations: Agostic interactions or not?

Joost M Bakker[1,2], Vivike Lapoutre[1], Andrew Sweeney[3], Abhigya Mookherjee[3], Peter B Armentrout[3]

[1]FOM Institute for Plasma Physics Rijnhuizen, The Netherlands, [2]Radboud University Nijmegen, The Netherlands, [3]University of Utah, Salt Lake City, USA

Keywords:
intracavity, transition-metal, agostic-interactions, carbene

Novel aspects:
This study provides the first IR spectroscopic characterization of transition metal carbene cations and reveal details of their bonding.

Abstract:
Methane, a key fossil fuel, is difficult to transport and difficult to convert to other energy rich chemicals because of the strong CH bond ; however, several third-row metal cations readily activate methane to form MCH 2 + + H2, a metal carbene. Except in one case, structures of these species have not been experimentally determined, although theory suggests several competing structures, some of which involve "agostic " interactions in which the electrons in a CH bond donate to the metal, distorting its structure. Transition metal carbenes are also interesting because they act as heterogeneous catalysts in the Shell higher olefin reaction that produces plasticizers and detergents. The low density of states in these four atom species means that efficient dissociation using multiple infrared (IR) photons requires high powers, made accessible recently by the Free Electron Laser for IntraCavity Experiments (FELICE) at the FOM Institute.

Ta, W, Ir, and Pt rods were ablated with a frequency doubled YAG laser (532 nm) to create gas phase atomic metal ions. These ions are entrained by a pulse of He gas containing methane gas to form metal carbene cations, MCH 2 +. Ionic species are irradiated by FELICE and the frequency of the light is varied from 300 to 3400 cm-1. Absorption of multiple IR photons can occur at frequencies corresponding to vibrational modes of the metal carbene cations, leading to dissociation of the molecule either by H or H 2 loss. Fragments and reactant ions are detected and mass analyzed using time-of-flight mass spectrometry (TOF MS) .

The IR multiple photon dissociation (IRMPD) action spectrum of TaCH 2 + (loss of H) is consistent with the calculated spectra for the 3 A' ground state of the carbene, TaCH 2 +. Calculations indicate this species has an "agostic " interaction, such that the molecule no longer has the expected C 2 v symmetry. No evidence for a hydrido-carbyne (HTaCH+) species is observed. The IRMPD spectrum of WCH 2 + (loss of H) is dominated by contributions from the ground state WCH 2 + (4 A ") , which has NO agostic interaction. Some evidence for contributions from a low-lying HWCH+ (2 A') species is possible. The IRMPD spectrum of IrCH 2 + (loss of H 2) is consistent with the calculated spectrum of the ground state species, HIrCH+ (1 A) with possible contributions from IrCH 2 + (3 A) structures, which have no agostic interactions. The IRMPD spectrum for PtCH 2 + (loss of H) is consistent with that calculated for the PtCH 2 + (2 A 1) ground state structure. This spectrum shows clear evidence of an overtone band at ~2000 cm-1. This late metal carbene cation has NO agostic interaction, consistent with no empty orbitals on the metal.

The high power afforded by the intracavity operation of FELICE allows efficient IR photodissociation of the transition metal carbene cations, species with a low density of states that would otherwise make them incompatible with IRMPD measurements. Such high powers can also complicate the spectrum, however, by introducing overtone bands.

Oral Session

Wedensday, 19th September

Room B-1

15:00 – 17:00

Session 28: IR Spectroscopy of Gas-phase Ions

Chair: Dietmar Kuck (Bielefeld University, Germany)

S28-1600

16:00 – 16:20

Gas-Phase Structure and Chemistry of the Radical Cation of DNA base-pair dGdC

George N khairallah[1], Linda Feketeova[1], Bun Chan[2], Vincent Steinmetz[3], Philippe Maitre[3], Leo Radom[2], Richard A O'Hair[1]

[1]University of Melbourne, Melbourne, Australia, [2]The University of Sydney, NSW 2006, Australia, [3]Universite Paris Sud, Orsay Cedex, 91405, France

Keywords:
Nucleobases, radical cations, CID, IRMPD spectroscopy, Watson-Crick base pair

Novel aspects:
This work is a first combined mass spectrometry/ IRMPD spectroscopy study of a simple model for radical cations of the complimentary base pairs found in DNA

Abstract:
Radiation damage of living cells can cause potential biological mutations. It is agreed that the mostradiation sensitive biomolecule in living tissues is DNA, where damage can occur in either the nucleobase or the sugar moiety, leading to strand breaks and causing mutagenesis and cancer amongst others. Collision-induced dissociation (CID) of a metal-biomolecule complex has been previously used to generate radicals from biomolecules, where loss of the biomolecule from the complex proceeds via electron transfer to produce the radical cation. Here, we used this method to form the deoxyguanosine (dG) /deoxycytidine (dC) radical cation ($[dGdC]^{\cdot+}$). This latter represents a simple model for radical cation formation of the complimentary base pairs found in DNA. In order to determine its gas-phase structure, IRMPD spectroscopy was used in combination with theoretical calculations. IRMPD spectroscopy of mass-selected ions is now an established and powerful technique for the structural characterization of gaseous ions and has been proven to work well with biomolecules.

We have used a hybrid ion trap/ ICR mass spectrometer (Finnigan LTQ-FT). This instrument consists of an ESI ion source coupled to a linear ion trap (LTQ) and a FTICR cell for high resolution accurate mass measurements. Multistage CID experiments were used to study the unimolecular chemistry of $[dGdC]^{\cdot+}$. IRMPD spectroscopy was performed at the CLIO facility in Paris using a FEL in the spectral range 900-1800 cm^{-1} as well as an OPO/CO_2 laser in the spectral range 3100-3700 cm^{-1}. These lasers were coupled to either a 3D ion trap or an FTICR mass spectrometer. *Ab initio* calculations were also undertaken using the Gaussian09 program and geometries were optimized with the M06-2X/6-31G(d,p). Literature scaling factors were employed in the evaluation of the ZPVE and IR frequencies.

The Unimolecular CID study has revealed a rich gas-phase chemistry including radical migration. Hence, the dissociation of the radical dimer, $[dGdC]^{\cdot+}$, showed that the radical migrates from the guanine nucleobase to its sugar moiety. From this, it is possible to conclude that radical damage to the nucleobase can propagate to the sugar backbone, leading to the cleavage of the DNA strand. The structure of $[dGdC]^{\cdot+}$ was revealed via a combination of theoretical calculations and experimental gas-phase infrared (IR) spectroscopy. The results obtained provided proofs indicating a hydrogen bonded $[dGdC]^{\cdot+}$ dimer structure (i.e.; Structure of the Watson-Crick base pair). The suitability of the theoretical calculation method used, was further proven via the successful comparison of the IRMPD spectrum of a simpler molecule, i.e.; $[9\text{-methyl-guanine}]^{\cdot+}$,to its calculated minimum energy structures. Therefore, the details of the fragmentation mechanisms as well as the IRMPD spectra and calculated structures will be presented.

S28-1620

16:20 – 16:40

Infrared spectroscopic studies on isomerization reactions of ionized molecular clusters

Yoshiyuki Matsuda, Naohiko Mikami, Asuka Fujii

Tohoku University, Sendai, Japan

Keywords:

Infrared spectroscopy, cluster, vacuum-ultraviolet photoionization, isomerization

Novel aspects:

Ionization dynamics of molecular clusters has been studied with infrared spectroscopy and theoretical calculation. This spectroscopic approach has been developed by us and is based on the vacuum-ultraviolet photoionization detection.

Abstract:

Understanding of ionization dynamics of molecular clusters would contribute to provide molecular level insights into ionization and protonation mechanisms in various ionization methods. We have investigated isomerization reactions upon ionization of molecular clusters with infrared (IR) spectroscopy based on the vacuum-ultraviolet (VUV) photoionization detection. [1] This spectroscopy enables to observe IR spectra of size-selected neutral and cationic clusters, which correspond to precursors and products in the VUV one-photon ionization, respectively. Therefore, this technique is powerful to study ionization dynamics of clusters.

Ionization-induced isomerization of various protic/aprotic molecules and their hydrated clusters has been investigated by combining the IR spectroscopic approach and theoretical calculations. [1-3] It has been elucidated that the proton-transfer reactions occur between the hydrophilic groups without energy barriers in the ionization of the clusters of methanol, ammonia, and acetic acid. [1] The proton-transfer reactions from the hydrophobic groups such as an alkyl groups have also been found in the acetone dimer, hydrated acetone, and hydrated formamide. [2,3] The ionized molecules in these clusters tautomerize to the enol forms through the transfer reactions of an alkyl proton. In the case of the hydrated clusters of acetone and formamide, the catalytic action of a water molecule for the alkyl proton-transfer reaction has been demonstrated experimentally and theoretically. [3]

Recently, the cationic structures of diethylether and trimethylamine dimers have been analyzed through the IR spectroscopy. These results indicate that alkyl groups in the cations have high acidity. The acidity of the cationic alkyl groups as well as the alkyl proton-transfer reactions will be discussed with the spectroscopic and theoretical results.

[1] Matsuda et al. Phys. Chem. Chem. Phys. 11, 1279 (2009).

[2] Matsuda et al. Phys. Chem. Chem. Phys. 14, 712 (2012).

[3] Matsuda et al. Angew. Chem. Int. Ed. 49, 4898 (2010).

Oral Session

Wedensday, 19th September

Room B-1

15:00 – 17:00

Session 28: IR Spectroscopy of Gas-phase Ions

Chair: Dietmar Kuck (Bielefeld University, Germany)

S28-1640

16:40 – 17:00

IR-MPD investigation of steric constraints on hydrogen bonding in dicarboxylic acid ions

Christine Merkert, Fabian Menges, Yevgeniy Nosenkov, Gereon Niedner-Schatteburg

TU Kaiserslautern, Germany

Keywords:
IR-MPD, hydrogen bonding, Eigen cation, Zundel cation, molecular switch

Novel aspects:
Steric constraints by twisting covalent bonds modulates proton transfer such that a model system for an isolated molecular switch emerges, elucidated by ab intio calculations and by IR-MPD spectroscopy

Abstract:
When isolated the protonated and deprotonated dicarboxylic acid ions HOOC-$(CH_2)_n$-C$(OH)_2^+$ and HOOC-$(CH_2)_n$-COO^- stabilize in cyclic form through ionic hydrogen bonding. Other than e.g. in hydrogen bonded clusters the alkyl back bone causes steric constraints which are expected to vary by the alkyl chain length. Ab initio calculations [1] predict a possible coexistence of symmetric (Zundel- type) and asymmetric (Eigen-type) hydrogen bonds in the case of adipic acid (n=4). A multitude of transition states define a stepwise proton transfer reaction path in concert with an equally stepwise twisting of the alkyl chain. We present Infra-Red-Multi-Photon-Dissociation (IR-MPD) spectra of dicarboxyilic acid cations and anions n=1,.., 6 and discuss fingerprints of proton localization and / or delocalization within the hydrogen bond. The importance of an appropriate temperature control will be emphasized. An outlook towards molecular switching will be provided.

[1] S.K.Min, M. Park, N. J. Singh, H. M. Lee, E. C. Lee, K. S. Kim, A. Lagutschenkov, and G. Niedner-Schatteburg ; Chemistry - a European Journal (2010), 16 (34), 10373-10379

Oral Session

Wedensday, 19th September

Room D

15:00 – 17:00

Session 29: The Advances in Biological Mass Spectrometry in Drug Discovery and Development: Current State of the Art and Challenges

Chair: Ajai K Chaudhary (Merck and Company, USA)

S29-1500

15:00 – 15:40

[Keynote Lecture] Advances in the Application of Mass Spectrometry to Drug Metabolism and Pharmacokinetics Studies

Ragu Ramanathan[1,2]

[1]Bristol-Myers Squibb, [2]Department of Biotransformation, Princeton, New Jersey 08540, USA

Keywords:
Drug Metabolism, Pharmacokinetics, Metabolites, Quantitation, Qual/Quant, UHPLC-Q-TOF

Novel aspects:
Invited keynote talk providing an overview of current applications of liquid chromatography-mass spectrometry (LC-MS) for various drug metabolism and pharmacokinetics (DMPK) studies.

Abstract:
The focus of this keynote talk will be to present an overview of current applications of liquid chromatography-mass spectrometry (LC-MS) for various drug metabolism and pharmacokinetics (DMPK) studies. Discovering highly potent and highly target selective new molecular entities (NMEs) are essential to develop efficacious and safe drugs for patients. Across the biopharmaceutical industry, LC-MS based techniques are utilized for many purposes by DMPK scientists due to their sensitivity, selectivity, specificity, and speed. Over the years, several mass analyzer types have been used in DMPK studies, including quadrupole mass filter (QMF), quadrupole ion trap (QIT), linear ion trap (LIT), time-of-flight (TOF), and Fourier transform mass spectrometers (FTMS).
Some of the desired qualities of the individual mass analyzer types and ion manipulation techniques have now been combined into a single instrument, generally referred to as hybrid mass spectrometers. Hybrid mass spectrometers have become very selective and specific when either a TOF or FTMS based high resolution mass spectrometer (HRMS) is combined with either ITs or QMFs. The ability of LITs and QMFs to operate in the vacuum pressure range of 10^{-5} to 10^{-6} torr makes these types of mass spectrometers ideally suited for coupling with LC separation and atmospheric pressure ionization (API) techniques as well as with TOF and FTMS based mass analyzers, in tandem, for capitalizing on the high resolution capabilities of the later two mass analyzers. Fast scanning HRMS based mass analyzers provide the option to integrate qualitative and quantitative analysis of drugs and their metabolites, under ultra high pressure liquid chromatography (UHPLC) conditions, and facilitate an increased throughput and streamlined workflow. These technological advances may allow a "paradigm shift" in how MS is used for various DMPK assays within the pharmaceutical industry.

Oral Session

Wedensday, 19th September

Room D

15:00 – 17:00

Session 29: The Advances in Biological Mass Spectrometry in Drug Discovery and Development: Current State of the Art and Challenges

Chair: Ajai K Chaudhary (Merck and Company, USA)

S29-1540

15:40 – 16:00

Development of stable isotope labeling by essential nutrients in cell culture (SILEC) coupled with LC-MS for drug target discovery

Ian A Blair, Nathaniel W Snyder, Zinan Zhou, Andrew Worth, Sankha S Basu

University of Pennsylvania, Philadelphia, PA 19104, USA

Keywords:

LC-MS, stable isotopes, neurodegenerative disease, CoA, lipids

Novel aspects:

SILEC methodology coupled with LC-MS has revealed potential new targets that can be employed for the development of drugs for treating neurodegenerative diseases.

Abstract:

Although mechanisms underlying selective cell death in neurodegenerative diseases such as Parkinson's and Alzheimer's disease, Friedreich ataxia, and amyotrophic lateral sclerosis are not well defined, mitochondrial complex 1 dysfunction has long been implicated. Coenzyme A (CoA) and CoA thioester derivatives are central players in numerous mitochondrial metabolic pathways. However, the lack of isotopically labeled CoA standards has limited the implementation of rigorous LC-MS-based methods for their analysis. We have developed stable isotope labeling by essential nutrients in cell culture (SILEC) methodology to generate stable isotope labeled CoA and thioester analogs for use as internal standards in LC-MS-based assays. This methodology can also be used to generate other stable isotope metabolite standards (such as menadione, an inducer of oxidative stress) so that accurate quantification of CoA species can be conducted. Much of the research on mitochondrial dysfunction has focused on induction of reactive oxygen species (ROS) and reactive nitrogen species generation. While these reactive species undoubtedly play a role in mitochondrial dysfunction by inducing macromolecular damage as well as by serving as signaling molecules, the source of the ROS and their exact role remains controversial. Using stable isotope dilution LC-MS, we have now shown that inhibition of complex 1 does not initially induce oxidative stress but causes a dramatic decrease in levels of succinyl-CoA and increase in levels of acetyl-CoA. By using [^{13}C] -labeling and isotopomer analysis, we have characterized the metabolic derangement in lipid metabolism that occurs. This has revealed potential new targets that can be employed for the development of drugs for treating neurodegenerative diseases. Supported by NIH grants T32HL007439 and P30ES013508.

Oral Session

Wedensday, 19th September

Room D

15:00 – 17:00

Session 29: The Advances in Biological Mass Spectrometry in Drug Discovery and Development: Current State of the Art and Challenges

Chair: Ajai K Chaudhary (Merck and Company, USA)

S29-1600

16:00 – 16:20

High-resolution metabolic profiling towards G-protein coupled receptors: rapid and comprehensive screening of histamine H4 receptor ligands

Jeroen Kool[1], Anders F Rudebeck[1], Frank Fleurbaaij[1], Saskia Nijmeijer[1], David Falck[1], Rogier Smits[2], Henry F Vischer[1], Rob Leurs[1], Wilfried M Niessen[1]

[1]VU University, Amsterdam, The Netherlands, [2]Griffin Discoveries B.V., The Netherlands

Keywords:
Correlation MS data functional activity

Novel aspects:
Identity with bioactivity correlation in metabolic mixtures. Bioactivity assessed as ligand binding selectivity and functional activity. Most important : correlation to identity of individual metabolites with accurate MS and MS/MS analysis.

Abstract:
In the past years, we developed high-resolution screening platforms involving separation of bioactive mixtures and on-line or at-line bioassays for a wide variety of biological targets with parallel mass spectrometric detection and identification. In the current research, we make a major step forward in the development of at-line bioassays by implementation of (functional) cell-based and membrane receptor binding assays. We demonstrate a new platform for high resolution metabolic profiling of lead compounds for functional activity and selectivity toward the human histamine H4 receptor (hH4R) , a GPCR. In this platform, a new post-fractionation cell seeding strategy, analytical chemistry, cell biology and pharmacology are merged. The methodology is based on chromatographic separation of metabolic mixtures by HPLC coupled to high-resolution fractionation onto (multiple) microtiter well plates for complementary assaying. Bioaffinity and selectivity were measured with radioligand displacement assays, while the functional assay was a gene reporter assay with adherent mammalian cells as pharmacological endpoint. The methodology was used for efficient and rapid metabolic profiling of three histamine hH4R selective lead compounds and the drug clozapine. Metabolites with undesired altered selectivity and/or functional activity profiles can be identified as well as bioactivation. The parallel structure identification of the metabolites, with accurate mass measurements and MS/MS, allowed identification of liable metabolic 'hotspots' for further lead optimization and plays a central role in the workflow and in this study. The methodology can be easily adapted for use with other GPCRs and GPCR screening formats. The efficient use of combining cell-based and binding assays with analytical techniques by leveraging high resolution at-line fractionation as a linking technology will allow implementation of comprehensive metabolic profiling earlier on in the drug discovery process.

Oral Session

Wedensday, 19th September

Room D

15:00 – 17:00

Session 29: The Advances in Biological Mass Spectrometry in Drug Discovery and Development: Current State of the Art and Challenges

Chair: Ajai K Chaudhary (Merck and Company, USA)

S29-1620

16:20 – 16:40

THE ROLE OF MASS SPECTROMETRY IN DRUG DISCOVERY AND DEVELOPMENT

ANTHONY TSARBOPOULOS

UNIVERSITY OF PATRAS, PATRAS, GREECE

Keywords:
UPLC Tandem Mass Spectrometry, Antibiotics, Protein PTMs, Noncovalent Interactions

Novel aspects:
Novel high-resolution UPLC-tandem MS methodologies for the quantification of cyclic peptide antibiotics. Monitoring of protein-ligand noncovalent interactions by ESI MS to shed light into disease processes.

Abstract:
Mass Spectrometry (MS) has played an important role in the design, discovery and development of new pharmaceuticals, from the earliest stages of disease determination through the final stages of clinical testing. Today, the whole effort is "target-driven " rather than the previously followed "disease-driven " method. Once a target has proven to be related to the disease process, high-throughput screening (HTS) methods may be used to determine initial structural leads among compounds generated from either total synthetic process, derivative of natural products, natural product etc. The selected compounds are then used for in vivo testing in preclinical species (mouse, rat, dog, monkey, etc.) . LC-MS and LC-MS/MS assays are the methods of choice for the late-stage discovery studies because they are rapid, sensitive, easy to automate and robust. In general, LC-MS and LC-MS/MS enters in all phases of drug discovery, i.e., phases I-III clinical trials, and especially providing an assessment of pharmacokinetics and drug metabolism prior to new drug application (NDA) . The members of the modern (bio) pharmaceutical industry are engaged in an on-going struggle to balance the needs for finding a new medicine while maintaining the relevant development cost in reasonable levels. The advent of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) methods combined with the rapid proliferation of several types of mass analyzers (especially quadrupole time-of-flight and orbitrap) has revolutionized the applicability of MS for studying proteins of high molecular weights by themselves or when they are bound to ligands. That, in turn, has opened the way to elucidate macromolecular structures and study their interactions with small molecules or other macromolecules, which are important aspects in understanding their biological function and developing new therapeutics. One of the main features of the aforementioned MS methods is their unique ability to determine whether the protein product presents any heterogeneity. This heterogeneity is usually associated with post-translational modifications (PTMs) , such as glycosylation, phosphorylation, proteolytic processing, deamidation, aggregation and formation of disulfide bonds, with all these contributing to the diversity of the protein product. Therefore, characterization of these protein molecules with regard to purity and structure is an essential and challenging task for protein therapeutics, and represents an integral part of the overall submission records to the regulatory agencies prior to protein's approval as a drug.
This presentation will outline the development and validation of a rapid, simple and accurate UPLC-ESI MS/MS analytical method on a hybrid qTOF instrument for the quantification of cyclic peptide antibiotics, which can be used in pharmacokinetic and metabolic studies of these drugs. In addition, complete structural analysis of recombinant protein pharmaceuticals and MS identification of PTMs, including identification of the glycosylation sites in a glycoprotein and structural elucidation of the attached oligosaccharide components will be illustrated. Finally, analysis of certain noncovalent interactions between bioactive molecules and proteins, which are responsible for certain diseases, will be demonstrated. Monitoring of these protein interactions in real-time by ESI MS will also provide the stoichiometry and the topology of the interacting species. This is nicely shown in the noncovalent complex detection of amyloid beta (Aβ) peptide with bioactive compounds. The Aβ peptide is currently believed to play a central role in the pathogenesis of Alzheimer's disease (AD) , and screening of bioactive antioxidants could lead to novel aggregation inhibitors for the prevention or treatment of AD. Overall, the elucidation of the structures and formation mechanism of these complexes could offer a better understanding of the disease processes, which in turn may aid in modern drug design.

S29-1640

16:40 – 17:00

Evaluation of fully automated DBS extraction system with LC-MS/MS systems for Sartans and Statins using different DBS card types

Souki Kanda[3], YASUHIKO BANDO[1,2], Takemoto Kawamukai[1], Hiroshi Hike[1], Amane Sakurai[3]

[1]AMR,Inc., [2]Biosys Technologies,Inc., Tokyo, Japan, [3]Toray Research Center, Inc., Kanagawa, Japan

Keywords:
Automated DBS extraction, High sensitive, Robust, Sartan, Statin

Novel aspects:
Fully automated DBS extraction systems overcomes the limitation of off line extraction method for quantitation of LC-MS/MS.

Abstract:

Introduction

The use of Dry Blood Spotting for sample collection gains popularity because of the low sample volume, handling and logistic benefits. It has been evaluated in a few years for the use in PK and TK studies, however, the reproducibility and sensitivity remains as an issue with current off-line punching and manual extractions. In addition, the three commercially available DBS cards have different characteristic resulted in the variation of extraction and quantitation of compounds to be analysed. In this study we have evaluated the fully automated extraction of compounds from DBS card and LC-MS/MS in order to overcome the exisiting limitations and validated the analytical method including drug stability on the cards for PK and TK application.

Methods

DMPK-A, DMPK-B, DMPK-C cards (GE healthcare) were evaluated with 8 compounds, 6 Sartans and 2 statins in rat whole blood (15ul) . The fully automated DBS extraction system (SCAP systems) integrated with LC-MS/MS (Agilent1100/4000QTrap) were utilized and evaluated in the aspect of compatibility of compounds to the type of cards. In addition quantitation with the internal standard method, reproducibility, drug stability on the card, sensitivity, reproducibility of Internal standard and carryover are examined based on Valsartan on Dry Blood Spot DMPK-A card.

Preliminary Data

Due the pretreatment of DMPK-A and DMPK-B card, the extraction efficiency varied from compound to compound. In general, DMPK-C card gained better extraction efficiency because of no pretreatment on the paper. No extraction took place on Simvastatin for all DMPK-A, DMPK-B and DMPK-C cards. The degradation or strong adsorption onto the card can be considered, however, further investigation will be required. Quantitation of valsartan from rat whole blood on the DMPK-A card gave excellent linearity from 10 - 10,000 ng/mL with 0.9987 correlation coefficient (r) under the internal standard method. The varsartan with 4 different concentration was spotted on the card and analysed in the same day. Accuracy (%) and CV (%) were both within 15% (n=5) and S/N of 10ng/mL (LLOQ) was >5 although the extraction area is only 1.5mm i.d.. The good reproducibility and sensitivity were achieved under fully automated systems. 15ul spot of whole blood with Varsartan, 20ng/mL (LQC) and 8000ng/mL (HQC) on DMPK-A card were packed with a plastic bag with desiccant and stored at ambient temperature for 14 days to see the stability. As a result of quantitation analysis, accuracy were both within ± 15%. Injection of internal standard were examined in the same analytical batch (n=35) and found CV (%) = 7.14. Ratio of peak area of 2 compounds, Varsartan and Losartan on DMPK-A card were calculated in order to see reproducibility of 4 extraction (1.5mm i.d.) within 1 blood spot. Variance was less than 15%. The reproducibility among spot 1~4 resulted in less than 15 % as well. 2 trap column switching system for the extraction of the compounds of interest reduced ion suppression. Extra washing mechanism reduced carry over significantly. Fully automated extraction system integrated with LC-MS/MS systems overcome the limitation of current off line extraction. The result shed the light on the use of DBS for PK and TK application.

Oral Session

Wedensday, 19th September

Room E

15:00 – 17:00

Session 30: Data Processing and Informatics for SIMS

Chair: DaeWon Moon (Center for Nano-Bio Technology, Korea Research Institute of Standards and Science, Korea)

S30-1500 [Keynote Lecture] Image and Spectral Processing for ToF-SIMS Analysis of Biological Materials

15:00 – 15:40

David G Castner
University of Washington, Seattle, USA

Keywords:
ToF-SIMS, multivariate analysis, image processing

Novel aspects:
Latest advances in multivariate analysis processing of ToF-SIMS image and spectral data will be presented. Proper correction and display of 3D images will also be presented.

Abstract:
Modern time-of-flight secondary ion mass spectrometry (ToF-SIMS) instruments can rapidly produce an enormous amount of complex data. Each spectrum typically contains hundreds to thousands of peaks. Each pixel in a 2D image contains a complete spectrum, which means for each 256 x 256 pixel image a total of 65,536 spectra are collected. When extended to 3D imaging the number of spectra increase by the number of 2D images acquired during the depth profile. Thus, millions of spectra can be acquired in a 3D image. This presents a significant challenge for the processing of ToF-SIMS image and spectral data. A powerful method for extracting information from the data sets is to use multivariate analysis (MVA), either in a supervised or unsupervised mode. The unsupervised mode uses all peaks in the data set, while the supervised mode uses a subset of the peaks (e.g., amino acid fragments for analyzing protein samples).

Principal component analysis (PCA) is one of the most common MVA methods used to process ToF-SIMS data. It determines which combination of original variables (i.e., peak intensities) account for variation across the data set. For example, it can be used to show that variation of different primary ion beam species (e.g., monoatomic vs. cluster) results in larger changes in the amino acid fragmentation pattern than the variation in amino acid content of different proteins (e.g., albumin vs. fibrinogen) [1]. It can also be used to discover the presence of unexpected species such as trace photoresist residue on a polymer film [2]. However, PCA results can depend on how the data is normalized and scaled, so it is often beneficial to do further processing with other MVA methods such as discriminant analysis or maximum autocorrelation factors (MAF). MAF has the advantage that it is scaling independent and can be applied to images from a range of different samples [3]. Filtering or preprocessing the image data prior to PCA processing can improve the PCA performance [4].

An additional challenge of 3D imaging is correcting the inaccuracies in the ToF-SIMS data that arise from projecting data from a curved surface onto a 2D image plane. Software has been developed (ZCorrectorGUI) that allows the entire 3D data cube to be properly corrected and displayed [5]. This software along with other MVA software for processing of ToF-SIMS data is available for free download at http : //mvsa.nb.uw.edu/.

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Oral Session

Wedensday, 19th September

Room E

15:00 – 17:00

Session 30: Data Processing and Informatics for SIMS

Chair: DaeWon Moon (Center for Nano-Bio Technology, Korea Research Institute of Standards and Science, Korea)

S30-1540 Multivariate analysis application to ToF-SIMS data of organic layers

15:40 – 16:00

Yasuko Kajiwara[1,2], Satoka Aoyagi[2,3]

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Keywords:
ToF-SIMS, Multivariate analysis, Organic layers

Novel aspects:
Effectiveness of multivariate analysis techniques such as multivariate curve resolution for complex Tof-SIMS data was investigated.

Abstract:
Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been widely used in the analysis of surface chemical structures of organic materials due to its high surface sensitivity and high chemical specificity [1] . However, it is often difficult to interpret complicated ToF-SIMS data of complex samples which include polymers. Recently, multivariate analysis (MVA) techniques have been applied to ToF-SIMS data analysis because they provide helpful information for interpreting ToF-SIMS spectra and images of complex samples [2, 3] .

In this study, principal component analysis (PCA) and multivariate curve resolution (MCR) after being preprocessed with Poisson-scaling were compared to evaluate a cross-section surface of organic layers. Organic layers were composed of three different polymers and the cross-section surface was mirror-polished with an ultra-microtome. ToF-SIMS spectra and images were acquired with a Ga ion source, and 216 peaks of secondary ions ranging from m/z 10 to 200 were employed to MVA.

In PCA, principal components could not properly extract the information of each polymer. Even though data with auto-scaling and no-scaling were also evaluated by PCA, the polymers were not clearly classified either. It was assumed that PCA missed subtle differences between similar materials when there were other dominant factors such as sample topography.

On the other hand, images of the three polymers were clearly separated by MCR and the spectra of these three components were similar to the reference spectra of the three polymers. Moreover, in the comparison with no-scaling, Poisson-scaled data results provided clearer images than no-scaled data results since Poisson-scaling relatively enhanced weak peaks which were characteristic to each polymer.

In conclusion, it is indicated that MCR is useful to extract pure components from mixed ToF-SIMS spectra of the organic composites. Moreover, minor differences such as functional groups among similar materials can be distinguished by MCR.

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Oral Session

Wedensday, 19th September

Room E

15:00 – 17:00

Session 30: Data Processing and Informatics for SIMS

Chair: DaeWon Moon (Center for Nano-Bio Technology, Korea Research Institute of Standards and Science, Korea)

S30-1600

16:00 – 16:20

The Full Spectrum protocol: how to provide quantitative analysis for matrix elements in heterogeneous nanometer scale layers with ToF-SIMS

Matthieu Py[1], Jean-Paul Barnes[2], Jiro Matsuo[1,3]

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Keywords:
ToF-SIMS, matrix effect, depth profiling, data treatment, full spectrum

Novel aspects:
The proposed Full Spectrum data treatment enables accurate, quantitative depth profile of matrix elements in heterogeneous matrix layers of nanometer scale with a commercial ToF-SIMS, which is usually impossible.

Abstract:
We propose to present on a theoretical and practical point of view a data treatment protocol for Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) that we recently updated, the so-called Full Spectrum protocol [1-4]. The latter, although relatively unknown and scarcely represented in the literature, is interesting because it capitalises on the specificity of ToF-SIMS (parallel monitoring of numerous secondary ions in one large range mass spectra). It indeed consists in taking into account as much information as possible from the ToF-SIMS mass spectra by following all of the secondary ions containing the element of interest, in contrast to all other approaches which usually focus on no more than one or two secondary ions. Precisely, for a given element *A*, one will sum the contributions of all $A_xR_y^-$ secondary ions normalised by x and by their isotopic abundances to obtain a new quantity corresponding to the atomic quantity of element *A* in the secondary ion beam. Instead of depth distributions of secondary ions intensities, the depth profiles are transformed into depth distributions of normalised sums of secondary ion intensities, somewhat equivalent to envelope functions, which are then calibrated to give quantitative depth profiles.

As a result, this approach shows better statistics and is found to be more representative of matrix element depth distribution in heterogeneous stacks. Another feature of this approach is that the signals it yields for matrix elements is directly proportional (although not equal) to the material composition. This approach presents therefore the advantage of enabling quantification for all matrix elements in one depth profile. Furthermore, since this ability only results from data treatment and not from instrumental or experimental conditions changes, one simultaneously keeps the ability of ToF-SIMS to quantify impurities or dopants if present. Due to the increased statistics, the quantitative profiles obtained through this protocol are also more reliable, reproducible and less noisy. Furthermore, for the particular case of very thin (<10 nm) samples, it was shown that this protocol enabled acquisition of quantitative profiles without resorting to reference samples (through cross-characterisation with X-ray Photoelectron Spectroscopy measurements).

In this work, the different aspects of the specific data acquisition and treatment for the Full Spectrum protocol will be discussed. First the theory behind the data treatment will be addressed. The improvements it brings in terms of reduction of undesired effects such as surface transients or matrix effects will be presented through simple, model samples as well as through structures for advanced microelectronics. Practical implementation issues will also be treated. Finally, limitations of the protocol will be quickly overviewed.

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S30-1620

16:20 – 16:40

Evaluation of secondary ions from lysozyme and peptides using G-SIMS and g-ogram

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Keywords:
G-SIMS, g-ogram, protein fragmentation, lysozyme

Novel aspects:
Complex fragment ions from a protein on ToF-SIMS spectra were characterized and clearly separated from secondary ions related to other organic materials by G-SIMS and g-ogram.

Abstract:
The characterization of proteins and peptides at surfaces is crucial for developing medical and diagnostic devices, bio-sensors and a tissue imaging technique. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is powerful for such purposes owing to its femto mole sensitivity, specificity between proteins, sub-micron imaging and 3D imaging. Since the surface information depth (approximately 1 nm) is smaller than the typical dimension of a protein it has been shown to give orientation specific information. Fragments from proteins relating to the functional group of each amino acid are mainly observed because it is difficult to generate intact protein ions, which makes interpretation of ToF-SIMS spectra of complex samples including proteins and peptides difficult. In addition, it is also difficult to characterize secondary ions and to distinguish target peaks from peaks from substrate materials and contaminants especially at a high mass range due to overlapped peaks.

Gentle-SIMS (G-SIMS) and g-ogram provide information on fragmentation of secondary ions based on ratio of static-SIMS spectra obtained with two primary ion conditions causing different amounts of fragmentation. The g-ogram is based on G-SIMS which is an effective method to simplify spectra based on the comparison between spectra having lower fragmentation and those having higher fragmentation. When the low fragmentation spectrum is multiplied by the ratio of the lower fragmentation spectra to higher ones then the peak intensities of the more intact, structurally significant, fragments are enhanced and the more degraded fragments suppressed in a G-SIMS spectrum. The g-ogram is an image representation for the G-SIMS spectrum normalized to the maximum intensity and suggests the fragmentation energetics of the ion formation like a traditional chromatogram (where the separation parameter here is the fragmentation energy) .

In this study, G-SIMS and g-ogram were applied to protein and peptide samples in order to investigate effectiveness of G-SIMS on characterization of proteins or peptides. Lysozymeor (des-tyr) -enkephalin on silicon wafer samples were measured using TOF-SIMS with Bi^+ and Mn^+ primary ion sources, and then peaks of secondary ions were analyzed by G-SIMS and g-ogram. As a result, molecular ions of secondary ions of (des-tyr) -enkephalin and secondary ions specific to lysozyme were clearly separated from other secondary ions related to substrates or organic contaminants by G-SIMS and g-ogram analysis. Some of the indicated protein-related secondary ions could be from dipeptide or tripeptide parts though it is generally difficult to find dipeptide or tripeptide peaks because they are often very weak and hidden by dominant peaks. Thus, G-SIMS and g-ogram analysis unveils intricate relationships between secondary ions to identifyt hem appropriately, and is useful to identify unknown peptides with co-existing organic materials and to characterize orientation or surface structure of a protein.

S30-1640 Ab initio peak identification for SRM/MRM data

16:40 – 17:00

Ken Aoshima[1], Koikegami Shigeru[3], Fukuda Mitsuru[3], Takahashi Kentaro[1], Matsuura Kentaro[1], Watanabe Hideki[1], Sato Yoshiaki[1], Uehara Taisuke[1], Kimura Takayuki[1], Nakamura Tatsuji[1], Parry Howell[2], Tanaka Satoshi[2], Utsunomiya Shin-ichi[2], Kajihara Shigeki[2], Tanaka Koichi[2], Oda Yoshiya[1]

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Keywords:
Targeted quantitative proteomics, MRM, peak detection, peak alignment

Novel aspects:
High accurate and large scale quantitation for SRM/MRM data

Abstract:

Introduction

Selected Reaction Monitoring (SRM) or Multiple Reaction Monitoring (MRM) is a powerful technique for proteomics analysis ; it can selectively quantify each specific fragment pair of precursor and product. There are several software tools already developed such as Skyline[1], MRMer[2] and Pinpoint software[3] for MRM based targeting quantitative proteomics analysis. However, some software tools can only quantitate SRM/MRM data based on MS/MS spectral libraries that were built in-house or publicly available, or can only perform relative quantitation using stable isotope labeling. In order to fully take advantage of SRM/MRM it is highly desired to develop an application tool which can perform absolute quantitation without the help of mass spectral libraries.

Method

Our application called 'EMS' derived from freeware software 'Mass++' is now equipped with a set of functions for *ab initio* peak identification of SRM/MRM data. These functions have been developed from those used in liquid chromatography mass spectrometry (LC/MS) data analysis :

1) The peak detection function finds a two-dimensional mixed Gaussian function which best fits to an MS chromatogram after the convergence process by expectation-maximization (EM) algorithm or by Levenberg Marquardt (LM) algorithm (also known as the damped least-squares method) . Before starting the convergence process, the function guesses the expected values of every component of the mixed Gaussian function as initial parameters based on k-means clustering. The number of components of the mixed Gaussian function should be selected by the Bayesian information criterion (BIC, also known as Schwarz criterion) or Akaike information criterion (AIC) to avoid over-fitting.

2) Dynamic programming (DP) and locally weighted scatterplot smoothing (LOWESS) are exploited in the peak alignment function.

Result

We have evaluated a variety of peak shapes of peptides obtained from different separation systems, using different MS data formats. Our peak detection algorithm can not only recognize the peaks identified by other software tools with help of mass spectral database/libraries, but can also properly detect the peaks which could not be extracted from mass spectral databases/libraries. This means that our algorithm can be useful for quantitative analysis of peptides which have not been registered in existing spectral databases/libraries. We have successfully analyzed over 80 complex proteomics data sets using our *ab initio* peak detection and alignment algorithms. All data sets were manually confirmed, proving that this algorithm was capable of analyzing large scale complex biological samples. Our *ab inito* peak detection and alignment algorithms will be integrated as one of the plug-ins for Mass++ and will be freely available on the Mass++ distribution site ;
http : //www.first-ms 3 d.jp/english/achievement/software

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PWe-001
11:10 – 12:20

Negative ion tandem mass spectrometry of prenylated fungal metabolites and their derivatives

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Keywords:
meroterpenoids, boviquinones, fungi, Basidiomycetes, LC- (-) -ESI-MS/MS

Novel aspects:
characterization of prenylated fungal metabolites and their derivatives by liquid chromatography negative ion electrospray tandem mass spectrometry

Abstract:
Meroterpenoid quinones occur widespread in various living organisms including macromycetes. They are natural products of combined biosynthetic origin which are partially derived from terpenoids [1]. A group of structurally related 2,5-dihydroxy-1,4-benzoquinones with prenyl side chains has been isolated from different fungal fruiting bodies belonging to the Boletales s.l. (Basidiomycetes) such as boviquinone-3 and boviquinone-4 including the related tridentoquinone formed by an unusual intramolecular ring closure [2, 3]. These metabolites were investigated by liquid chromatography electrospray tandem mass spectrometry under negative ionization. A detailed study of the fragmentation behavior of various prenylated benzoquinones and phenols from different fungal species was carried out by using an ion trap system.
The results obtained can be summarized as following :
· Prenylated benzoquinones and phenols exhibit a characteristic fragmentation pattern in negative ion electrospray tandem mass spectrometry.
· The mass spectral decomposition of both prenylated phenols and boviquinones under negative ion ESI conditions is mainly characterized by typical successive losses of the isoprene units. This is in agreement with their corresponding EI mass spectra [4, 5].
· The loss of the isoprenoid side chain of the boviquinones leads to a common radical anion comprising the quinoid moiety.
· In contrast to the linear boviquinone-4, the cyclic tridentoquinone shows a quite different fragmentation pattern.
· In case of the prenylated phenols, the loss of the complete isoprenoid side chain gives also information about the substitution pattern of the benzyl type moiety.
In conclusion, the loss of the whole side chain as well as the characteristic neutral loss of a single isoprene unit provides useful information both structural features of the meroterpenoids.
The obtained mass spectral data were applied to a classification of several fungal species of different genera belonging to Boletales s.l. by a principal component analysis (PCA).

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PWe-002
13:30 – 14:40

Metabolome profiling of human embryonic stem cells by gas chromatography-mass spectrometry

Takashi Suzuki[1], Masahiro Miyoshi[2], Katsuhiro Nakagawa[1], Hirofumi Suemori[3], Shinichiro Chuma[3], Norio Nakatsuji[3]

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Keywords:
Biomarker, GC-MS, hESCs, metabolomics

Novel aspects:
This is the first study to perform metabolome profiling of human embryonic stem cells by gas chromatography-mass spectrometry

Abstract:
Human embryonic stem cells (hESCs) have the potential not only to proliferate indefinitely but also to differentiate into any cell type in the body. These properties make hESCs promising for applications such as drug testing, disease modeling, and cell replacement therapy. Development of techniques for their quality control and stable supply is indispensable for the above applications. For this purpose, effective biomarkers that are able to evaluate the quality of hESCs should be established. Although there are many studies of characterization of pluripotent stem cells in genomics, transcriptomics, and proteomics, a few efforts have been made in metabolomics. The aims of our study are to establish effective metabolite biomarkers for quality evaluation of hESCs and to develop a simple and quantitative method for measurement of the biomarkers using mass spectrometry (MS) . In this study, we analyzed differences of metabolic signature of hESCs in the undifferentiated and differentiated state using gas chromatography-MS (GC-MS) . Two lines of hESCs, KhES-1 and KhES-3, were analyzed. We observed about 50 peaks in the spectra of oxime-trimethylsilyl derivatives of cellular metabolites. We observed about 10 metabolites which show more than two-fold difference between differentiated and undifferentiated hESCs. We also analyzed total cellular fatty acids. GC-MS profile in undifferentiated hESCs showed differences from that in differentiated counterparts. These results suggest that metabolomic technique is one of the effective strategies to discover biomarkers for evaluating the undifferentiated state of hESCs.

PWe-003
11:10 – 12:20

Metabolic characterization by mass spectrometry of Cordyceps bassiana mycelium cultivated under various culture medium and light conditions

Sun-Hee Hyun[1], Seok-Young Lee[1], So-Hyun Kim[1], Gi-Ho Sung[2], Seong Hwan Kim[3], Hyung-Kyoon Choi[1]

[1]College of Pharmacy, Chung-Ang University, Seoul, Korea, [2]Mushroom Research Division, Department of Herbal Crop Research, National Institute of Horticultural & Herbal Science, Suwon, Korea, [3]Department of Microbiology, Dankook University, Cheonan, Korea

Keywords:
Cordyceps bassiana mycelium, Metabolic characterization

Novel aspects:
Metabolic characterization of C. bassiana mycelium cultivated under various conditions and optimum culture conditions for the production of target metabolites.

Abstract:
Metabolic alterations of ***Cordyceps bassiana***mycelium were investigated under the following culture medium and light conditions: dextrose agar supplemented with 0.5 % yeast extract (SDAY) medium with light (SL) , SDAY medium without light (SD) , nut medium without light (ND) , and iron-supplemented SDAY medium without light (FD) . The growth of mycelia was retarded under the ND condition compared to the other conditions. The levels of asparagine, aspartic acid, glutamic acid, glutamine, histidine, lysine, ornithine, and proline were significantly higher under SD and SL conditions. The levels of most of the alcohols, saturated fatty acids, unsaturated fatty acids, fatty acid esters, sterols, and terpenes were higher under the ND condition than in the other conditions, but beauvericin was not detectable under the ND condition. The FD condition was favorable for the enhanced production of aminomalonic acid, malic acid, mannonic acid, and erythritol. Thus, the metabolic characteristics of *C. bassiana* can be manipulated by varying the cultivation conditions, rendering this fungus potentially favorable as a nutraceutical and medicinal resource.

PWe-004
13:30 – 14:40

Electrochemical simulation of Phase I and II Drug Metabolism, A Powerful Technique for Metabolite Characterization and Synthesis

Jean-Pierre Chervet[1], Agnieszka Kraj[1], Hendrik-Jan Brouwer[1], Nico Reinhoud[1], Martin Eysberg[1], Uwe Karst[2]

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Keywords:
Metabolite synthesis, drug metabolism, electrochemistry

Novel aspects:
A long-lasting metabolite synthesis by applying square wave pulses

Abstract:
Electrochemistry (EC) in combination with mass spectrometry creates a powerful platform to simulate various oxidation and reduction processes in life sciences. Electrochemistry is a complementary technique to traditional *invivo* or *in vitro* metabolism studies, and delivers the oxidative metabolic fingerprint of a (drug) molecule in a very short time. Mass spectrometry delivers selective and sensitive detection and allows for unambiguous identification of all products generated in the electrochemical cell. Additionally, automated data analysis by use of data bases (proteomics) or e.g., mass spectral trees (metabolomics) can shorten considerably the total time needed for the experiment.

In this poster a dedicated electrochemical system will be presented for fast screening of drugs to obtain their metabolic fingerprint. The easy and fast electrochemical conversion of Amodiaquine into its major phase I metabolites will be presented in both analytical and preparative EC cells. In a second step Glutathione (GSH) is added to the electrochemically generated metabolites to form the appropriate GSH-metabolite adducts, mimicking phase II reactions. All known adducts were successfully formed and identified with MS. Investigation of drug-protein adducts by conventional techniques (microsomal incubation, in-vivo studies) are very laborious and time-consuming. With the application of EC, it is possible to activate proteins and drugs within seconds to undergo covalent drug-protein binding. The conjugation of ß-Lactalbumin with Amodiaquine metabolites will be shown.

A new method for highly efficient metabolite synthesis based on a square-wave potential pulses will be presented. The stable oxidation conditions were obtained without the need of any cell maintenance for a prolonged period of time.
Verapamil was chosen as model drug and its oxidation products and metabolites were investigated. A highly concentrated sample (250uM) of the Verapamil was used for the experiments and electrochemically synthesized metabolites are collected off-line. Mass spectra are measured to confirm the presence of Verapamil metabolites in the collected samples.

Additionally, the applicability of on-line EC/MS to oxidize other drugs and xenobiotics (e.g., acetaminophen, irinotecanetc.) will be presented. The data demonstrate that hyphenation of electrochemistry with ESI/MS provides a versatile and user-friendly platform for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) in phase I and phase II metabolism studies.

PWe-005
11:10 – 12:20

Identification of a new plasma biomarker of Alzheimer Disease using metabolomics technology

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Keywords:
Alzheimer's disease, Biomarker, LC/APCI-MS, Desmosterol

Novel aspects:
Plasma desmosterol could be a powerful new specific biomarker for the early and easy Alzheimer Disease diagnosis.

Abstract:
Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system (CNS) characterized by a progressive loss of short-term memory accompanied by a gradual loss of cognitive functions. Biomarkers are very useful for diagnosing and monitoring disease progression and are important for patient selection, monitoring side-effects, aiding to select appropriate patient treatment and helping new drug discovery. For the clinical studies of AD therapeutics, there is an increasing need for diagnostic markers to ensure that therapies are targeted at the right patient population, to initiate early treatment when disease-modifying drugs will be available and to monitor disease progression. Several studies have investigated AD biochemical biomarkers in various tissues including blood and CSF. Of those approaches, CSF Ab x-42 and tau protein phosphorylation are currently considered the most useful biomarkers, though they cannot predict conversion from MCI to AD accurately and are not useful for guiding drug treatment. In addition, analyzing those markers requires performing delicate CSF collection from patients. Hence, access to less invasive biomarkers found in easy to acquire fluids such as plasma would accelerate and reduce the cost of AD diagnosis and offer windows of opportunity for selecting and treating patients with disease-modifying drugs once they are available.

Herein, we performed unbiased analysis of steroids related compounds to identify novel AD plasma biomarkers using LC/APCI-MS and Mass ++ data analysis software[1)]. The analysis revealed that Desmosterol, a cholesterol precursor, was found to be decreased in AD plasma vs healthy elderly controls plasma (n=10) with a fold change and p value of 0.36 and less than 0.01, respectively. In order to precisely quantify variations in desmosterol between normal controls and AD patients, we established an analytical method to measure desmosterol and cholesterol concentration using LC/APCI-MS system that allowed separating 5 endogenous main isomers and interfering peaks. Using this LC-based method, we discovered that desmosterol and desmosterol/cholesterol ratio are significantly decreased in AD patients using this LC-based method. Those changes were not observed in samples from Parkinson's disease (PD) and schizophrenia patients samples, suggesting the specific association of desmosterol with Alzheimer's disease and or with cognitive decline. Next, the validation of this assay using 109 clinical samples confirmed the decrease of desmosterol in AD patients as well as a change in desmosterol/ cholesterol ratio in AD patient's plasma. Interestingly, we could also observe a difference between control and Mild Cognitive Impairment patients (MCI) . In addition, the decrease of desmosterol was somewhat more significant in female patients. Receiver operating-characteristic (ROC) analysis between controls and AD, using plasma desmosterol shows a score of 0.80, indicating a good discrimination power of this marker in the two reference populations and confirms the potential usefulness of measuring plasma desmosterol levels for diagnosing AD. For control versus MCI, the ROC value using plasma desmosterol is 0.71, suggesting a more moderate discrimination power. Moreover a significant correlation of plasma desmosterol with CSF Ab x-42/Ab x-40 and Mini-Mental State Examination (MMSE) scores, suggesting that desmosterol/cholesterol ratio might be reflective of disease progression.

Our studies demonstrate a sensitive and accurate method of detecting plasma desmosterol concentration and suggest that plasma desmosterol could be a powerful new specific biomarker for the early and easy AD diagnosis.

Reference

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PWe-006
13:30 – 14:40

Metabolic profiling of mouse brain with MALDI-FT-ICR imaging mass spectrometry technique

Kazunori Saito[1], Daisuke Miura[2], Fujimura Yoshinori[2], Nirasawa Takashi[1], Wariishi Hiroyuki[2]

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Keywords:
Metabolomics, Imaging MS, FT-ICRMS

Novel aspects:
MALDI-FT-ICR imaging MS can reveal the distribution of the various metabolites without tandem MS technique in tissue sample

Abstract:
Matrix assisted laser desorption ionization (MALDI) -based imaging mass spectrometry (MS) is a superb technique to understand the distribution of the various molecules in tissues. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is thought to be one of suitable detection technique for the small molecules, such as drugs and metabolites, due to its ultra-high resolution. This ultra-high resolution (typically ~500,000 FWHM in small molecules) of FT-ICR MS can resolve not only for separation of the closed mass of different molecules but also so-called resolved isotopic fine structures, which can allow more confident identification of the elemental composition. Here we apply MALDI-FT-ICR imaging MS to detect metabolites in ischemia mouse brain.

Ischemia-induced mice were used as a model animal. Normal and ischemia mouse brain tissue were sectioned at 10um thickness and placed on ITO-coated glass slides. A matrix-solution of 5 mg/mL of 9-aminoacridine in MeOH was applied to the sample by an airbrush. A Bruker Daltonics FT-ICR MS solariX was used for imaging mass spectrometry experiment. The instrument equipped with the 7 tesla superconductive magnet and ESI/MALDI dual ion source. The ion source has a dual ion funnel and a quadrupole. The generated ions were accumulated in a hexapole to enhance the sensitivity. Ions were injected into ICR cell through the hexapole ion guide. Image was generated on flexImaging software and spectra were analyzed on DataAnalysis software.

MALDI with 9-aminoacridine matrix can detect many metabolites such as nucleotides and phosphorylated sugars. Those species were revealed in MALDI-imaging MS as well. Ultrahigh spectral resolution of FT-ICR MS could resolve closed peaks, e.g. only 13 mDa differentiated peaks of deprotonated cAMP anion (328.04524) and sodium-adducted glutathione anion (328.05847) . In addition, two mass imaging for these two compounds could be generated, and spatial distribution of both compounds in the tissue was separately shown. These facts show that mass spectra acquired in MALDI-imaging MS were potentially intricate and this level of high resolution is essential for imaging MS of the metabolites or any other small molecules from the tissue sample. Furthermore, high resolution spectra can allow direct identification of the metabolites without tandem MS technique, and elemental compositions were clearly identified from the mass spectra. The formula identification was strength by comparing the obtained spectrum with the theoretical one which showed isotopic fine structures.

In this study, spatial distribution of the metabolites were analyzed by FT-ICR imaging MS without tandem MS. More than 20 of the metabolites were detected and analyzed by one acquisition of the imaging MS. Ultrahigh spectral resolution was required to separate complicated peaks and peak assignments. It was concluded that FT-ICR MS is one of suitable platform for imaging MS of the metabolites from the tissue sample.

PWe-007
11:10 – 12:20

Ultra Fast Analysis of Amino Acids in Cultured Cell Extracts Using UHPLC/MS/MS

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Keywords:
amino acids, fast analysis, UHPLC/MS/MS, cell extracts, dipeptide

Novel aspects:
Ultra fast analysis of amino acids using UHPLC/MS/MS was applied to cultured cell extracts. As a result, low concentrations of amino acids were detected without severe interference from the matrix.

Abstract:
An essential aspect for the research of metabolic behavior involves the analysis of amino acids, which is typically carried out using ion-exchange high performance liquid chromatography (HPLC) with post-column derivatization using o-phthalaldehyde or ninhydrin. Recently, not only relatively high concentrations of amino acids like proteinogenic amino acids but only relatively low concentrations of amino acids and related compounds like non-proteinogenic amino acids and dipeptides are also monitored as significant targets for this purpose. In case of biological samples, however, interferences by other compounds which involve high levels of amino acids significantly affect the sensitivity for low concentrations of amino acids due to low selectivity of this analytical method. Moreover this method requires over two hours for the trace analysis of amino acids including related compounds. Because of these problems of traditional method, many kinds of methodology which aimed at higher selectivity or faster analysis time have been investigated. Highly selective and sensitive analysis of amino acids can be achieved using mass spectrometry. Using reversed-phase ultra high performance liquid chromatography (RP-UHPLC) techniques remarkably developed in recent years with pre-column derivatization can shorten analysis time drastically. Herein, we describe the trace analysis of amino acids and related compounds in biological samples using UHPLC/MS/MS with pre-column derivatization, along with its application for the analysis of cultured cell extracts.

Samples were prepared and derivatized using the EZ：faast amino acid kit (Phenomenex) and analyzed using a reversed-phase core-shell type column Kinetex C18 (Phenomenex) and a UHPLC system (Shimadzu Nexera) coupled with a triple quadrupole type mass spectrometer (Shimadzu LCMS-8030) . Using our analytical methodology, 36 amino acids and related compounds were detected selectively with MRM and LOQ were ranged from 2 to 500 pmol/mL. Moreover the total analysis time using this method was significantly shorter than that of traditional HPLC methods - our method requires 7 minutes for sample cleanup / derivatization, and 7 minutes for the chromatographic analysis.

The applicability of our methods for biological samples was demonstrated using cultured cell extracts of human colon cancer cell and human fibroblast cell. As a result, pmol/mL range of amino acids like prolylhydroxyproline, carnosine and aminoadipic acid were detected from human colon cancer cells as isolated peaks with the selectivity of triple quadrupole mass spectrometer besides coexistence of nmol/mL range of amino acids like glutamine, glycine and alanine.Also in the case of human fibroblast cells, any severe interference in peaks of amino acids which exist at relatively low concentrations was not confirmed. Recovery test was also carried out for 8 amino acids which were detected at low concentration in each sample. Recovery ratio for those amino acids were in the range from 80 to 120 percent about both human colon cancer cell and human fibroblast cell and it indicates the matrix in cultured cell extracts didn't cause severe suppression effect nor enhancement effect.

Those satisfactory results show that the UHPLC/MS/MS with pre-column derivatization can be applied to biological samples as ultra fast analysis in particular for low concentrations of amino acids and related compounds.

Investigations are currently underway to expand our methodology using other biological samples.

PWe-008
13:30 – 14:40

Untargeted screening of pesticides metabolites by LC-HRMS: a tool for human exposure evaluation?

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Keywords:
Pesticides, screening, untargeted, HRMS, metabolomics

Novel aspects:
The semi-targeted method developed in this work allowed to distinguish various groups of individuals according to their exposure to pesticides, on the basis of several urinary metabolites.

Abstract:
The estimation of human exposure to pesticides still represents a challenge since sample amounts available from a human cohort study are often very low, and the search for possible compounds has to be as thorough as possible. From urine samples, pesticides are generally detected as metabolites whereas all possible metabolites structures could be unknown. In this context, this work aimed at the assessment of an untargeted approach using UHPLC-HRMS to characterize pesticides metabolites in urine samples from the "PELAGIE " (endocrine disruptors : longitudinal study on pregnancy anomalies, infertility and childhood) human cohort study. The PELAGIE study was drawn to evaluate the consequences of the exposure to multiple contaminants, and particularly to determine the influence of the exposure to pesticides on pregnancy, birth and psychomotor growth of the child. This study was conducted on a representative cohort of 3421 pregnant women living in a French rural area (Brittany) .
In our work, 40 samples were randomly selected from 4 groups of individuals variously exposed to pesticides on the basis of their environment : urban population versus rural population surrounded by more or less cereal cultures. These samples were directly analyzed by UHPLC-HRMS (stationary phase C18, Electrospray ionization in the positive and negative mode, LTQ-Orbitrap mass spectrometer) . Obtained data were processed with the MetWorks software (Thermo Scientific) to extract and integrate HRMS signals of 47 pesticides and their known or theoretical metabolites. Moreover, a major advantage of this approach is the possible detection of compounds which are not present in the initial metabolites list. Up to now, almost 450 substances (pesticides + putative urinary metabolites) were monitored by this way. Following their detection by UHPLC-HRMS, MS^n experiments were performed to confirm or not, the detected compounds as potential or probable metabolites. Some of them have also been confirmed by comparison with metabolites generated during a parallel animal experimentation.
From human samples, 24 metabolites were identified using ESI in the negative mode and integrated. Data obtained by ESI in the positive mode could only confirm the identification of the metabolites detected in the negative mode, but did not allow the characterization of new compounds. Data were then processed by PLS-DA after an OSC filtration. The best separation of samples groups was obtained by the data normalization with the use of an internal standard during UHPLC-HRMS experiments. According to the model generated by this way, the separation of individuals was explained by 6 variables representing 3 pesticides, among which 2 fungicides classically used in cereal cultures were found.
The semi-targeted method developed in this work allowed to distinguish various groups of individuals according to their exposure to pesticides, on the basis of several urinary metabolites. The acquisition of full scan HRMS signals allows having complete datasets available to setup targeted MS/MS experiments for the structural identification of the metabolites detected in a first attempt.

PWe-009
11:10 – 12:20

Metabolite profiling of Makgeolli with Different Wild Yeast Strains Saccharomyces cerevisiae 98-5 and Pichia anomala 197-13 During Fermentation

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Keywords:
metabolite, makgeolli, Saccharomyces cerevisiae, Pichia anomala

Novel aspects:
Metabolite profiling of Makgeolli (traditional Korean alcoholic beverage) is reported for the first time.

Abstract:
Makgeolli, which is also called *takju*, is a traditional Korean alcoholic beverage. It is made by steaming glutinous rice, non-glutinous rice, barley, or flour with *nuruk*, yeast and water. After fermentation for 7 days, it contains about 15-16% alcohol content. When drinking, water is added so that the alcohol content of *makgeolli* is lowered to around 6-7 %. In this study, temporal changes in the metabolites of *makgeolli* by using wild yeast strains *Saccharomyces cerevisiae* 98-5 and *Pichia anomala* 197-13 during fermentation were analysed by ultra performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF MS) and gas chromatography. The resultant data were statistically processed by partial least squares-discriminant analysis (PLS-DA) and principal component analysis (PCA). Various metabolites, including amino acids, organic acids, fatty acids, small peptides, and urea cycle intermediates were obviously altered by increasing the fermentation period. Changes in these metabolites permitted discrimination among *makgeolli* samples with different wild yeast strains and fermentation periods (1, 2, 3, 6, 7, and 8 d) on a PCA score plot. The sensory attributes of the *makgeolli* used in this research had higher scores than 5-point of 9-point hedonic scale with anchors (1-extremely dislike, 5-neither dislike or like, 9-extremely like).

PWe-010
13:30 – 14:40

Plant metabolite analysis using comprehensive two dimensional gas chromatograph quadruple mass spectrometer

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Keywords:
GCxGC-QMS, High scan speed, 20,000u/sec, Metabolomics, Plant

Novel aspects:
We developed GCxGC coupled with a quadruple mass spectrometer (QMS) featuring high scanning speed up to 20,000 u/sec and utilized for the analysis of plant metabolites.

Abstract:

Introduction

The various metabolites at different concentration levels are analyzed simultaneously in metabolomics field. GC-MS is one of the most widely used techniques. However, the separation capability is not sufficient. Comprehensive two dimensional gas chromatograph mass spectrometer (GCxGC-MS) is promising method for improving the separation.

Even though time of flight mass spectrometer (TOFMS) is commonly used as the mass spectrometer of GCxGC-MS which requires high scan speed, the dynamic range of TOFMS is insufficient to cover a wide concentration range for plant metabolites.

We developed GCxGC coupled with a quadruple mass spectrometer (QMS) featuring high scanning speed up to 20,000 u/sec and utilized for the analysis of plant metabolites.

Methods

The work present here was performed by GCMS-QP2010 Ultra (Shimadzu, Japan) equipped with GCxGC modulator (Zoex, US) . A 30 m long, 0.25 mm id capillary with a 0.25 μm thick stationary phase of 5% phenyl-95%-methylpolysiloxane (DB-5, J&W, USA) was used as the first-dimension column and a 2.5 m long, 0.10 mm id capillary with 0.10 μm thick stationary phase of 50% phenyl polysilphenylene-siloxane (SGE, Australia) was used as the second-dimension column. For compound identification, NIST mass spectral library and GC/MS metabolite mass spectral database (Shimadzu) were used.

Grained rice, Japanese millet and foxtail millet were prepared as evaluation samples. An extraction solvent consisting of a mixture of water/ methanol/ chloroform (1/2.5/1) and the heptadecane as internal standard were added to 30mg of the crushed sample. After thorough mixing and centrifugation, 900 µL of the liquid phase was withdrawn. Milli-Q water (400 µL) was added to the retained liquid phase to separate the mixture into 2 phases (water / methanol phase and chloroform phase) . After centrifugation, 400 µL of the water / methanol phase was removed. Next, application of partial vacuum and centrifugation were conducted to remove the methanol from solution, and the remaining liquid was freeze-dried. The freeze-dried residue was subjected to trimethylsilyl (TMS) derivatization ; and this derivatized sample was used for GC/MS analysis.

Results and Discussion

The samples were analyzed at scan speed of 20,000 and 10,000 u/sec, separately. The width of peaks was from 300 to 400 ms. The number of the sampling points across the peaks was around 5 to 6 at 10,000 u/sec and 10 at 20,000 u/sec. Since at least eight sampling points are necessary for precise chromatographic peak shapes for each peak, the scan speed of MS should be set to 20,000 u/sec. However, at higher scan speed, ion transmission is remarkably decreased in QMS. In order to overcome this problem relating to QMS, we optimized a DC voltage to be imposed on quadruple rods (Advanced Scanning Speed Protocol) . Using this technology, the sensitivity and quality of mass spectra at 20,000 u/sec were improved and the dynamic range was expanded to 1,000 without compromising the sensitivity. Using this system, the metabolites extracted from rice, Japanese millet and foxtail millet were analyzed. The number of detected metabolites was from 1950 to 2147 compounds after separating the over-lapping peaks in 1 st dimensional chromatograph. In rice sample, 2147 compounds were detected and 1988 compounds showed more than 600 similarity score by mass spectral library search. These results demonstrated the GCxGC-QMS can be effectively used to detect plant metabolites with a wide concentration range.

PWe-011

11:10 – 12:20

Differentiation of German and Roman Chamomiles by Comprehensive Chemical Fingerprinting using Ultra Performance Supercritical Fluid Chromatography Coupled

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Keywords:
Comprehensive chemical fingerprinting, UPSFC/MS

Novel aspects:
Comprehensive chemical fingerprinting of chamomiles using UPSFC/MS for the first time with pros/cons relative to other mature analytical techniques investigated.

Abstract:
Introduction

Natural product ingredient profiling is a challenging task due to sample complexity. The analyte diversity requires the utility of multiple analytical techniques to provide orthogonality which can best provide comprehensive knowledge of the analyte composition. Presently, a variety of analytical techniques exist (such as HPTLC, UPLC, GC, NMR etc) which cover a range of chemical space. Here, we use Chamomile as an application example to investigate and explore the advantages and limitations of a novel technology, the Ultra Performance SFC (UPSFC) with the end goal of obtaining a comprehensive ingredient profiling for Chamomile and gaining chemical understanding of the key ingredients that differentiated different Chamomiles (such as German, and Roman) . This UPSFC/UV/MS method was compared with our previous studies.

Methods

The Chamomile samples were extracted with methanol (UPLC/UV/MS, UPSFC/UV/MS) , and hexane (UPSFC/UV/MS) separately. For UPLC/UV/MS, a Waters ACQUITY UPLC with PDA and Xevo Qtof MS was used. The mobile phase was water with 0.05 % formic acid (A) , and acetonitrile (B) . Data acquisition was ESI+. For UPSFC, a Waters ACQUITY UPSFC/UV/SQ detector was used. A VIRIDIS Hybrid UPSFC column with 2.1 x 150 mm was used with carbon dioxide as mobile phase A, and methanol as mobile phase B.

Preliminary Data

Chamomile is often used for relieve symptom of sleeplessness, anxiety, and gastrointestinal conditions. The flowering tops of the chamomile plant are used to make teas, liquid extracts etc. Normally, Chamomile refers to either German chamomile or Roman chamomile, which are from the same family (Asteraceae) but belong to different genera. The main components described in chamomile flowers belong to the classes of volatile derivatives and flavonoid components. The analytical methods can be used for plant comparison studies including GC-MS, LC-UV-MS, and UPSFC/UV/MS, all have unique capabilities in identification and authentication of plant samples and commercial products. Use of all these techniques will be helpful for chromatographic fingerprint analysis of chamomile samples. Information obtained represents a comprehensive qualitative approach for the purpose of species authentication, quality control, ensuring the consistency and evaluation of their related commercial products. For UPSFC analysis, we took a systematic screening approach showing the impact of the column, mobile phase modifier, pressure, and flow rate on the packed column supercritical fluid separation. A simple 10 minute gradient method ramping 2 -40% co-solvent (s) was used to screen columns of cyano, flourophenyl, 2 -EP, C18 and hybrid silica stationary phases. A flow rate of 2 mL/min was used. The final chromatographic conditions are presently being optimized and will be presented. Coupling to the mass spectrometer was achieved by splitting the flow prior to entry to the photodiode array detector. Different methods optimizing the MS signal were explored. The compared approaches include direct flow to the MS versus the addition of a make-up flow to increase ionization versus applying heat to the flow stream prior to entry into the MS stimulating a phase change increasing solubility of the sample with the co-solvent. The UPSFC result will also compared with our previous results obtained from analytical technique (UPLC-UV-MS) .

PWe-012
13:30 – 14:40

Synthesis of 13C6- and 13C12-dabsyl chloride to perform amine-targeted clinical profiling

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Keywords:
dabsyl chloride, stable isotope, amine, amino acid, plasma

Novel aspects:
We have prepared dabsyl chloride with three diffrent mass to develop a method to perform accurate profiling of amines in clinical samples.

Abstract:
Dabsyl chloride (4-dimethylaminoazobenzene-4'-sulfonyl chloride) has a visible chromophore with very high extinction coefficient. Therefore, this reagent has been used to accurately quantify amines in various biological samples. To obtain the quantitative profiles of amines in clinical samples such as urine and plasma, it is necessary to perform high performance liquid chromatography coupled with UV-vis detection and mass spectrometry (HPLC-vis-MS) . However, due to the limitation of the mobile phase suitable for MS, base-line resolution of chromatography is difficult to attain. To overcome this problem, in the present study, we successfully synthesized 13C6- and 13C12-dabsyl chloride in gram order from 13C6-aniline. First, sulfanilic acid-13C6 and N,N-dimethylaniline-13C6 were synthesized from aniline-13C6 by treatment with sulfuric acid and methyl p-toluenesulfonate, respectively. Second, 13C6- and 13C12-methyl orange were produced by diazocoupling of sulfanilic acid-13C6 with N,N-dimethylaniline. Finally, methyl orange was treated with PCl5. By labeling sample of interest, control sample, and a mixture of standards with 13C0-, 13C6- and 13C12-dabsyl chloride, respectively, each amines is expected be detected as triplet peaks mutually separated by 6 mass. To confirm this, we derivatized a mixture of 18 amino acids by incubation with 13C0-, 13C6-, and 13C12-dabsyl chloride, respectively, at 70C for 10 min under vigorous stirring. These three labeled samples were mixed and then analyzed by HPLC-vis-MS) using a capillary C18 column and electrospray ionization. All of the amino acids could be detected as characteristic triplet peaks, and the completeness of the derivatization could be evaluated quantitatively.

PWe-013
11:10 – 12:20

Use of culture media fingerprinting by LTQ FT Ultra MS to predict human embryonic implantation potential

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Keywords:
Fingerprinting, metabolites, culture media, reproductive

Novel aspects:
This MS metabolites profile could help the selection of the most viable embryo, improving single embryo transfer and thus eliminating the risk and undesirable outcomes of multiple pregnancies.

Abstract:
Embryo selection methods based on morphologic parameters are usually associated with the Assisted Reproductive Technologies (ART) routine. However, most studies suggest that morphology evaluation is not enough to predict a genetically normal embryo and successful implantation. As the number of ART cycles increases worldwide, improvements in the ability to perform a non-invasive embryo viability prediction has become a crucial target for reproductive medicine. Mass spectrometry (MS) fingerprinting is a global chemical screening approach to compare and classify samples based on metabolite patterns, with the ultimate goal to identify discriminating ions, usually corresponding to metabolites. MS-fingerprinting uses direct sample infusion in the ionization source and usually just sample dilution is performed. This case-control study evaluated mass spectrometric (MS) fingerprinting patterns of culture media samples used for human embryo culture of patients undergo ingintracy to plasmic sperm injection (ICSI) with minimal sample preparation and minute-analysis to predict embryo implantation potential.
Culture medium used for the incubation of 85 embryos from 38 patients undergoing ICSI was collected after embryo transfer at Day 5. The remaining culture media were collected and samples were split into groups according to their implantation outcomes : 100 % of implantation (n=15, 8 patients) , 66.7 % of implantation, when 3 embryos were transferred but 2 embryos implanted (n=12, 4 patients) , 50 % of implantation, when 2 embryos were transferred and 1 implanted (n=16, 8 patients) , 33.3 % of implantation, when 3 embryos were transferred and only 1 implanted (n=21, 7 patients) and 0 % of implantation (n=21, 11 patients) . Samples were individually diluted and injected directly to the ESI source coupled to a MS. The samples (2 µL) were dissolved in 200 µL of methanol/water 4 : 1 (v/v) containing 0.1% ammonium hydroxide for analysis in the negative ion mode. Mass spectra fingerprinting were acquired using a 7.2T LTQ FT Ultra-MS equipped with a chip-based direct infusion nanoelectrospray ionization source operating in the negative ion mode at the follow conditions : a 200 nL/min flow rate, 0.3 psi backing pressure, and 1.5 to 2.0 kV electrospray voltages during 120 s, controlled by ChipSoft software. Mass resolution was fixed at 100,000 (defined for an ion of *m/z* 400) throughout. Data were obtained as transient files (scans recorded in the time domain) along the 100-1000 *m/z* range. MS datasets were exported using the Xcalibur 2.0 and organised by Markerlynx XS, where the ions relative intensities of each spectrum were considered. Data analysis was conducted in MatLab 7. 0 version using Partial Least Squares Discriminant Analysis (PLS-DA) toolbox.
There were 6,476 observed ions in the *m/z* range of 100 to 1000 after spectral noise reduction. The samples were divided into calibration and validation subsets. MS spectra from samples of 0 % and 100% of implantation were organize into calibration set (n=36) and spectra from 66.7%, 50% and 33.3% were mount into validation set (n=49) . The PLS-DA model described more than 92.2 % (y-block) of the data variance and was able to classify all of the calibration samples correctly. In the validation process, samples of the 66.7% group were correctly identified with 87.8% average probability : samples of the 50% and 33.3% groups were accurately classified with 100% average probability in this multivariate model.
The preliminary results show that implantation success of human embryos can be correlated with specific biochemical profile in their culture media. MS-fingerprinting by LTQ FT Ultra-MS is a fast, simple and non-invasive way to detect these patterns, which allied to the multivariate statistical model, was able to predict with a high confidence the potential of individually embryo implantation. This biochemical profile could help the selection of the most viable embryo, improving single embryo transfer and thus eliminating the risk and undesirable outcomes of multiple pregnancies.

PWe-014
13:30 – 14:40

Ionic liquid-based microextraction for sensitive determination of urinary aromatic amines by liquid chromatography-mass spectrometry

Jingyueh Jeng, Cheng-Yuan Shih

Chia nan University of Pharmacy & Science, Tainan, Taiwan

Keywords:
Room temperature ionic liquids, liquid chromatography-mass spectrometry, urinary aromatic amines, dermal exposure

Novel aspects:
Room temperature ionic liquid as a novel media to extract the urinary aromatic amines and sensitive determination of amines by liquid chromatography-mass spectrometry

Abstract:
Room temperature ionic liquids (RTIL) were used as the novel media for extraction and preconcentration of aromatic amine in rat urea. A suitable mixture of extraction solvent (250μL, 1-butyl-3-methylimidazolium hexafluorophoshate, [C_4MIM] [PF_6]) and dispersive solvent (500μL, methanol) were injected into the aqueous samples (4 mL), forming a cloudy solution. After centrifuging, enriched analytes in the sediment phase were determined by HPLC-MS/MS. Some important parameters, such as the kind and volume of extraction solvent and dispersive solvent, sample pH, sample volume and salt effect were investigated and optimized. Under optimum conditions, enrichment factors for 2,4- and 2,6-toluenediamine were above 50 and the limits of detection (LODs) were 2.7 and 3.4 ng/mL, respectively. Relative standard deviations (RSDs) were below 5.0%. Furthermore, the estimated percentage of recovery from amine-spiked urine samples ranged from 77 to 112%. The method presented here is simple and fast, and does not involve the use of volatile organic extractants.

PWe-015
11:10 – 12:20

Adjustment of total cellular metabolite concentration by flow-injection analysis mass spectrometer for metabolomic study

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Keywords:
FIA-MS, metabolomics, cellular metabolites, calibration, adjustment

Novel aspects:
This is the first method using FIA-MS to adjust the cellular metabolite concentration prior to metabolomic analysis

Abstract:
Metabolomic studies on cell biology have shown huge impact on drug discovery, cancer therapy, toxicology, and stem cell research in recent years. A general approach for genomics and proteomics study is to adjust the total amount of nucleic acid and protein for a fair comparison. However, in metabolomic studies, only cell number was controlled regardless of the difference in cell volume or cell types which could lead to a biased comparison. This study proposed a novel and reliable method to determine the total amount of cellular metabolite concentration through the evaluation of total ion chromatograms (TIC) obtained by flow injection analysis -mass spectrometry (FIA-MS) . Area under TIC was used to estimate cellular metabolite concentration. Parameters including data acquisition rate, FIA mobile phase composition, flow rate, injection volume, and MS parameter such as capillary voltage, sheath gas temperature and flow in Jet Stream electrospray ionization (ESI) , vaporizer temperature and corona voltage in atmosphere pressure chemical ionization (APCI) , were investigated for their effects on TIC of FIA-MS. Both ESI and APCI were compared for their performance on calibrating cellular metabolite concentration. Serial dilution of cell extracts was used to construct the calibration curve, and it was used to calibrate cellular metabolites concentrations in metabolomic studies. Our data indicated that using FIA-MS is effective to adjust cellular metabolite concentration before metabolic profiling of cell extracts.

PWe-016
13:30 – 14:40

Metabolomics of Curcuma: approaching by studying constituents of wide range polarity using LC-DAD-ECD-MS

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National Chiayi University, Chiayi, Taiwan

Keywords:
metabolomics, Curcuma, on-line assay, electrochemical detector, mass spectrometry

Novel aspects:
We developed a special ECD coupled diode array detection and electrospray ionization mass spectrometry for detecting molecules eluted from gradient HPLC mobile phase, involve from aqueous to non-aqueous.

Abstract:
The characteristic information, such as structure and antioxidation property of curcuminoids and polyphenolic compounds in different *Curcuma* species, or in the same species collected from different cultivation regions is critically important for understanding the metabolomics of the plant and which can be applied in medicine, new species breeding, or even quality control. However, there is no suitable on-line antioxidant assay method for analyzing the relatively non-polar and polar constituents in Curcuma. A lot of researchers have studied antioxidant activities and structure of antioxidant compounds. Meanwhile, variety of analytical methods was developed to meet the need of the activity and structure identification simultaneously. The devices designed for simultaneous antioxidant assay and structure identification are currently most promising for high throughput analysis. However, the application that published in activity assay of antioxidant compounds is still have many problems. Electrochemical detector (ECD) is one of simple and widely used method for antioxidant analysis. Application of ECD in detecting molecules of wide range polarity eluted from high performance liquid chromatography (HPLC) is limited by the poor solubility of electrolytes in non-aqueous mobile phase. In the present study, we developed a special ECD coupled diode array detection and electrospray ionization mass spectrometry for detecting molecules eluted from gradient HPLC mobile phase, involve from aqueous to non-aqueous. The device was applied to inquired antioxidants, including polar and nonpolar ingredients simultaneously in *Curcuma* species. We got signals of antioxidant activity response by applied of the ECD for qualitative analysis, chromophore absorbance of corresponding curcuminoid compounds in wavelength 425 nm and polyphenolic compounds in wavelength 280 nm by applied of diode array detector, and mass spectrometry for identifying curcuminoids and polyphenolic compounds from four Curcuma species in the eight different cultivation regions. The rhizomes of *Curcuma xanthorrhiza*, *Curcuma domestica*, *Curcuma aureginosa*, and *Curcuma heyneana* were analyzed, three major components : curcuminoid (1), demetoxycurcuminoid (2), and bisdemetoxycurcuminoid (3) have been identified, based on their electrochemical spectra, UV spectra, and mass spectra. The method was successfully in characterizing curcuminoids and polyphenolic compounds, their polarity are essentially opposing to each other. Hence, the present high throughput method can be used for studying metabolomics of *Curcuma* and other similar species, that containing secondary metabolites of diverse polarity.

PWe-017 Effect of glucose addition against ion suppression by sodium chloride

11:10 – 12:20

Megumi Wakimoto[1], Yui Okamura[1], Hajime Mizuno[1], Naohiro Tsuyama[1], Sachiko Date[2], Tsutomu Masujima[1,2]

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Keywords:
Ion suppression, ESI, nano ESI

Novel aspects:
We investigated ion suppression effect of NaCl and found moderating effect of glucose against suppression effect induced by NaCl.

Abstract:

[Introduction]

In the process of electrospray ionization, matrix molecules cause signal increase or decrease of target analytes, which is referred as "matrix effect ". For example, salts, which are included in biological samples a lot, cause signal suppression.

We have developed a nano ESI-based mass spectrometric method called "Live Single-cell Mass Spectrometry ", which can analyze small amounts of subcellular contents directly by a nanospray tip. Therefore, ion suppression effect can be a serious problem if it occurs in Live Single-cell MS even though nano ESI is one of the methods that enables to decrease the ion suppression effect.

In this study, by comparing offline nano ESI with conventional ESI, we investigated if nano ESI could prevent ion suppression effect. Also we examined how we could improve this problem.

[Method]

To analyze the ion suppression, sodium chloride (1-1000μM) or D-glucose (1-1000μM) was added to a mixture of nine amino acids (Ser, Thr, Leu, Asp, Glu, Met, His, Phe, Arg, 1 μM each) dissolved in 50% MeOH. We analyzed these samples by offline nano ESI and conventional ESI at positive ion mode. Spray voltage was 1.0kV in nano ESI and 4.5kV in conventional ESI. Flow rate in conventional ESI was 10.0μL/min. The experiments (n=6) were perfomed on the LTQ Orbitrap XL (Thermo Fisher Scientific) at the resolution power of 100,000. Nanospray emitters we used for these experiments were Cellomix Tips (HUMANIX, i.d. 3 μm) . We compared the peak intensities of amino acids among different condition. Furthermore we used RBL-2H3 cell lysate ($5.0x10^6$ cells/mL) to confirm glucose effect on ion suppression.

[Result]

By adding 1 mM NaCl to the amino acids mixture, protonated ion peaks of most amino acids disappeared in conventional ESI spectra, while they decreased to 50-80 % in nano ESI. This result showed that the application of nano ESI caused less ion suppression than conventional ESI. It seemed that nano ESI produced smaller droplets owing to its small internal diameter compared with conventional ESI. Size of droplets was one of the determinants of suppression effect by NaCl. Nano ESI could improve suppression effect but not completely.

In contrast, peak intensities of amino acids gradually increased by adding glucose. We hypothesized that glucose could remove sodium ion in the nanospray tip because of its high affinity to sodium ion.

Next, in order to remove suppression effect of NaCl we added glucose to sample solution. The peak intensities of amino acids in the presence of both NaCl and glucose were about twice as high as only with NaCl. On the other hand, sodium ion adducted amino acids peaks were hardly detected with glucose while it could be highly observed without glucose. It suggested that increasing sodium ion adducted glucose resulted in decreasing ion suppression because of sodium ion trapped by glucose molecules.

Moreover, we investigated this glucose effect was available to actual biological samples on enhancement of peak intensities of analytes. We measured RBL-2H3 lysate added with 1 μM glucose by nano ESI. As a result, peak intensities of most amino acids increased by glucose addition. For example, peak intensities of His and Arg increased about five times. Thus, glucose could increase peak intensities of analytes even in biological samples with complex composition.

Now, we examine if glucose addition enables sensitive detection of various molecules in Live Single-cell Mass Spectrometry.

PWe-018
13:30 – 14:40

Discriminant analysis of Curcuma species grown in different origins using mass spectrometry

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Keywords:
Discriminant analysis, Metabolite profiling, Mass spectrometry, Multivariate statistical analysis

Novel aspects:
There was a considerable change of the levels of metabolites such as essential oils and phenolic pigments depending on two Curcuma species grown in different origins.

Abstract:
Curcuma species is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae*. It has been used as a spice in curries and natural dye and investigated the effect as herbal medicines to treat some diseases such as Alzheimer, cancer and other clinical disorders. It has been also known that secondary metabolites such as essential oils and phenolic pigments in *Curcuma* species can have therapeutic actions in humans and which can be refined to produce drugs.

In order to investigate the metabolic difference of two *Curcuma* species in Jeju-do and Jin-do of South Korea, four groups of sampleswere grown for 5 months. Quantitative analysis of essential oils and phenolic pigments like curcuminoids in *Curcuma* species was performed using GC-TOF-MS and UPLC-TOF-MS, respectively.

Metabolite profiling coupled with multivariate statistical analysis was carried out to characterize the differences between species (C. aromatica and C. longa) or origins (Jeju-do and Jin-do) . PCA score plot showed significant differentiation among two *Curcuma* species, whereas there is no difference between Jeju-do and Jin-do. A *t*-test was performed to statistically certify the metabolite difference in metabolite levels. Differenciation between two *Curcuma* species was due to higher levels of fifteen metabolites such as eucalyptol, gemacrenediones and elemene and lower levels of seventeen metabolites shch as cedrene, curcumene, germacrone, tumerone and zingiberene in C. aromatica. The levels of Curcuminoids were also changed in two *Curcuma* species grown in different origins.

This study indicated that there was a considerable change of the metabolic pattern depending on two *Curcuma* species grown in different origins and mass spectrometry coupled with multivariate analysis is an efficient method to discriminate *Curcuma* species grown in different origins.

PWe-019
11:10 – 12:20

Copper Stress Induced Global Metabolites Profiling of Burdock Roots by 1H NMR, GC-MS and UPLC-QTOF MS Analysis

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Keywords:
Burdock Roots, Copper Stress, Metabolomics, GC-MS, UPLC-QTOF MS

Novel aspects:
GC-MS analysis showed the increase of fatty acids such as linoleic acid and linolenic acid in copper stress-induced burdock roots.

Abstract:
Arctium lappa L. (Asteracease) , known as burdock, has long been cultivated as a popular vegetable for dietary use and folk medicine worldwide, as a diuretic and antipyretic tea as well as hypertension, gout, arteriosclerosis, hepatitis and other inflammatory disorders. In the literature, many health benefits associated to occurrence of different classes of bioactive secondary metabolites have been reported. These classes include, among others, flavonoids and lignans, and burdock is an important natural source of compounds from both families.
Stress in plants could be defined as any change in growth condition (s) that disrupts metabolic homeostasis and requires an adjustment of metabolic pathways in a process that is usually referred to as acclimation.
Phytoalexins are low molecular weight antimicrobial compounds that are synthesized *de novo* and accumulate in plants under abiotic stress. Phytoalexins have been well documented in the field of plant defense. Primary bioactive compounds accumulated under abiotic stress may possess various health promoting benefits including antioxidant activity, anti-inflammation activity, cholesterol-lowering ability, and even anticancer activity.
Copper, one of the heavy metals, is a microelement necessary for plant growth. Generally, heavy metals activate the phenylpropanoid pathway and increase lignin synthesis in many plant species.
Metabolomics could significantly contribute to the studies of biological stress responses in plants and other organisms by identifying different compounds, such as by-products of stress metabolism, stress signal transduction molecules or molecules that are part of the acclimation response of plants.
In the present study, ^{1}H NMR, GC-MS and UPLC-QTOF MS have been developed for the detection of copper stress-induced metabolites in burdock roots. We could observe the changes of these metabolites by global metabolite profiling using ^{1}H NMR and UPLC-QTOF MS analysis. In particular, GC-MS analysis showed the increase of fatty acids such as linoleic acid and linolenic acid in copper stress-induced burdock roots. These data suggest that metabolomic approach using combined ^{1}H NMR, GC-MS and UPLC-QTOF MS analysis is an effective analytical method to understand metabolism about copper stress-induced metabolites in burdock roots.

PWe-020
13:30 – 14:40

The impact of high-resolution MS techniques on the discovery of novel natural products from myxobacteria

Yasuhiko Maekawa[1], Daniel Krug[2,3], Thomas Hoffmann[2,3], Aiko Barsch[4], Gabriela Zurek[4], Rolf Mueller[2,3]

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Keywords:
Identification of new econdary metabolites, high-resolution TOF mass spectrometry, combining targeted and untargeted profiling, metabolome-mining

Novel aspects:
Myxobacteria as showcase : a survey of current developments in high-resolution MS and database-assisted strategies enhancing the natural products discovery workflow.

Abstract:

Introduction

Myxobacteria represent an important source of novel natural products exhibiting a wide range of biological activities. Some of these so-called secondary metabolites are investigated as potential leads for novel drugs. Traditional approaches to discovering natural products mainly employ bioassays and activity-guided isolation, but genomics-based strategies become increasingly successful to reveal additional compounds. These “metabolome-mining “ approaches hold great promise for uncovering novel secondary metabolites from myxobacterial strains, as the number of known compounds identified to date is often significantly lower than expected from genome sequence information. High-resolution mass spectrometry plays a crucial role for bridging the apparent gap between genomic potential and secondary metabolome reality, by enabling a powerful combination of targeted and untargeted analysis.

Methods

Myxobacteria were cultivated in complex media and extracts were separated by RP chromatography using an U-HPLC system. MS measurements were performed in ESI positive and negative mode using an ultra high resolution Q-TOF mass spectrometer (scan range 100-2000 m/z) . A specialized database system -Myxobase- was developed to support targeted screening. Identification of known compounds was accomplished using precise EICs and considering exact mass, retention time and isotope pattern for increased confidence. Myxobase was also designed to host comprehensive spectral information following processing of raw data by molecular feature extraction. In addition, feature-extracted HR-MS data were used for untargeted profiling by Principle Component Analysis. MS/MS information confirmed compound identity or enabled evaluation of chemical novelty for compounds differentiating bacterial strains.

Results

We present here high-resolution MS applications which are aimed at the discovery of novel secondary metabolites from myxobacteria. The challenges encountered in these studies comprise the need for sensitive and highly reproducible analysis in combination with high resolution, accuracy and extended sample throughput. Using data from an up-to-date ESI-UHR-Q-TOF MS platform, the development of methods and tools which are key to success is discussed.

Targeted profiling :
Full scan spectra enable the targeted screening for known myxobacterial compounds based on highly selective EIC traces, using Myxobase as analyte database. Profiling reports are parsed into Myxobase with all relevant analytical performance qualifiers, enabling collaborative evaluation of results and crosslinking with bioactivity assays and strain-related data. Our screening campaign comprising more than 1000 samples allows for the first time the in-depth comparison of high numbers of myxobacterial secondary metabilte profiles and the evaluation of metabolite diversity across all myxobacterial species, based on extracts from a world-wide collection of myxobacteria.

Metabolome-mining :

Molecular feature extraction facilitates the deposition of comprehensive metabolite profiles inside Myxobase. Thereby, the secondary metabolomes of all analyzed myxobacteria become backwards-searchable for the presence of novel natural products. This feature enables the straight-forward identification of alternative producers, an important achievement since newly identified secondary metabolites are frequently produced in low yields only. Moreover, the comparison of feature-extracted secondary metabolomes using statistical tools has the potential to uncover previously "hidden " natural products. This untargeted "metabolome-mining " approach is exemplified by the recent discovery of myxoprincomides, an intriguing family of novel secondary metabolites from Myxococcus.

Identification :
Spectral matching to a reference library of high-resolution MS 2 data from myxobacterial compounds is used to confirm the identity of known metabolites. In addition, high-resolution tandem-MS measurements are a crucial prerequisite for assessment of putative chemical novelty and serve as a valuable starting point for structural elucidation.

PWe-021
11:10 – 12:20

Differential analysis of fermented beverage using fast polarity switching TOFMS acquisition with high mass accuracy and multivariate analysis

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Keywords:
Profiling study, Electrospray, Time of Flight, MSn, Structure Determination

Novel aspects:
Profiling study using polarity switching and MSn data acquisition without compromising mass accuracy on fermentation product research

Abstract:
The metabolomics technique can rapidly bring information about the similarities and differences within a chromatographic dataset. A metabolomic based approach has been established for metabolite profiling and biomarker discovery. However, it is equally applicable to other research fields including industrial chemical product characterization, food analysis and natural product research. In the case of fermented product research, sample profiling is often a significant challenge due to the intrinsic differences between samples influenced by the combination of ingredients, production processes, and storage conditions. In this study, we developed an LC-based approach to determine metabolite profiles including polar metabolites and to identify specific endogenous components, aiming at high throughput and comprehensive methods using TOFMS acquisition.

Commercially available beers were used as a test sample for the polar metabolite analysis. All UHPLC units are consisted of Nexera LC-30A series (Shimadzu Corporation) with a pressure range up to 130 MPa. The chromatographic system used a binary solvent system delivered as a gradient of water and acetonitrile containing 0.1% formic acid, and used a Synergi Hydro-RP column (Phenomenex). The accurate full-scan MS measurement was carried out on a Shimadzu LCMS-IT-TOF mass spectrometer with rapid polarity switching. Profiling Solution software (Shimadzu) was used to create a data array for all sample data ; this tool was used to highlight specific components that were statistically different and to export data to SIMCA-P+ (Umetrics) for PCA and PLS-DA analysis.

Beer is a beverage made by fermentation from malted barley and flavored with hops, and the existence of many organic compounds in it is known. These compounds show ion signals in both positive and negative ionization (organic acid preferentially ionizes in negative mode, whilst nucleoside shows higher signal intensities in positive mode). To obtain the complete profile from a sample, it is necessary to run the LC/MS analysis both positive and negative modes. In preliminary experiment, seventeen products of commercial beer and related beverage were analyzed in triplicate by LCMS-IT-TOF systems using ESI with a scan range of *m/z* 80 to 1000. Synergi Hydro-RP column could separate the polar metabolites such as organic acid, nucleobase and nucleoside under water-rich conditions. Profiling Solution software was used to transform LC/MS raw data into an aligned data array of retention time and m/z pairs. 912 peaks were detected by peak extraction function from positive mode and 528 peaks in negative mode, within 20 min of LC separation. To determine metabolite profiles, principal component analysis was performed with thousands of unique retention time and m/z pairs. By performing the PCA, four experimental groups classified according to the malt ratio (classification by Liquor Tax Law of Japan : beer, low-malt beer and the third beer) were roughly classified into three clusters. Profiling software and PCA was used not only to establish the differences between the two conditions but also to highlight specific components that could account for the PCA data. The ion of *m/z* 191.0199 in negative mode and *m/z* 205.0976 in positive mode was extracted as a characteristic peak of high-malt beer. These peaks were tentatively assigned as citric acid ($C_6H_8O_7$) and tryptophane ($C_{11}H_{12}N_2O_2$) respectively, using formula prediction software that takes into account mass accuracy, isotope modeling and MS^n information. These were further identified by comparison with authentic standards. When mass accuracy was checked with the known compound such as malic acid and adenosine, it turned out that MS measurement was performed in the accuracy of less than 3 ppm (using external calibration) acquired with fast polarity switching.

In conclusion, PCA of LCMS peak profiles of beers resulted in the separation of samples according to some of their different compositional properties.

PWe-022

13:30 – 14:40

Simultaneous Quantitative and Qualitative measurements for Primary Metabolism Investigations using a Quadrupole-Time-of-Flight Mass Spectrometer

Noriyuki Iwasaki[1], Don Richards[3], Mark Savage[4], Angus Nedderman[4], Carsten Baessmann[2]

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Keywords:
DMPK, QTOF, metabolite, quantitation

Novel aspects:
Demonstration of simultaneous quantitative and qualitative DMPK analyses using Q-ToF technology at 1 uM drug concentrations.

Abstract:
Recently there has been considerable interest in simultaneously performing both quantitative and qualitative DMPK analyses in support of small molecule drug discovery. This work describes an investigation into the possibility of using a Quadrupole-Time-of-Flight mass spectrometer to obtain clearance data, metabolite identification, structure elucidation and metabolite profiles from P450 microsomal incubations at a drug concentration of 1 mM from a single sample set.
P450 microsomal incubations of commercially available drug substances including Pindolol, Verapamil and Haloperidol were prepared at 1 mM concentration. The incubations were sampled and quenched, at intervals to provide a time course over a period of 60 minutes. The analysis of the samples was carried out by LC-MS using a Bruker Maxis Impact Quadrupole-Time-of-Flight mass spectrometer to obtain data suitable for measuring clearance and plotting metabolic profiles. Data dependent MSMS spectra were collected in order to identify and elucidate the structures of the observed metabolites. The chromatographic method used a generic gradient, reverse phase method based on a Kinetex C18 column and 0.1 % formic acid and acetonitrile mobile phases at a flow rate of 300 ml /min.
Preliminary data showed that Pindolol was not cleared. The clearance values obtained for Verapamil and Haloperidol were $t_{1/2}$ = 7 and 47 minutes respectively. These were as expected and comparable to the values obtained on the same sample set using a triple quadrupole mass spectrometer.
In the case of Verapamil two metabolites, Norverapamil and an N-dealkylation product were easily observed by comparison of base-peak chromatograms and their structures determined through examination of the MSMS data. A third metabolite was uncovered using metabolite detection software and although the response was considerably weaker, it's structure was determined in the same manner. The LC-MS data were re-processed to plot the profiles of the three metabolites.
No metabolites of Haloperidol were immediately apparent but two were uncovered using the metabolite detection software. These were found to be oxidation of the piperidine moiety to a pyridine and an N-dealkylation product. Again, the LC-MS data were re-processed to plot the profiles of these metabolites.
The ability to carry out all of these analyses at 1 mM concentrations and under generic conditions indicates that improved workflows for simultaneous DMPK measurements can be developed to greatly enhance the information yield and productivity of both quantitative and qualitative determinations. A refined methodology and work flow will be fully described in the poster presentation together with further examples.

PWe-023

11:10 – 12:20

Development of accelerate quantification analysis for hydrophilic metabolites using ionparing chromatography with a high-speed triple quadrupole mass spectrometer

Hashim Zanariah[2], Yudai Denpo[2], Tairo Ogura[1], Ichiro Hirano[1], Takeshi Bamba[2], Eiichiro Fukusaki[2]

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Keywords:

Targeted metabolomics, Non-volatile compounds, Hydrophilic metabolites

Novel aspects:

High throughput analysis technique of hydrophilic metabolites using ion-pare chromatography with a high-speed MRM analysis were developed.

Abstract:

Metabolomics is the comprehensive study of metabolites, and it allows for detailed phenotypic analysis through a combination of metabolite information. Among the many kinds of metabolic reactions, central pathway of energy metabolism is one of biologically important pathway, and it includes around a hundred of hydrophilic compounds such as sugar phosphates, organic acids, and nucleotides. Ion-pare chromatography coupled to a triple quadrupole mass spectrometer is one of the techniques to analyze these hydrophilic metabolites. On the other hand, in order to improve throughput of analysis, the mass spectrometer which combines high-speed with a quantitative capability is needed. In this study, we report a developed analytical system for hydrophilic metabolite using ion-pare chromatography with a high-speed triple quadrupole mass spectrometer.

Standard samples of each metabolite were analyzed to optimize conditions of liquid chromatography and mass spectrometer. And the cellular extracts were analyzed as test samples. Samples were measured by electrospray triple quadrupole mass spectrometer (LCMS-8030, Shimadzu) coupled to UHPLC system (Nexera series, Shimadzu) . Analytical conditions were as follows, column : L-Column ODS (2 mmI.D. x 150 mmL., 3 micrometer) ; flow rate : 0.3 mL/min ; column temperature : 40C ; mobile phase A: water containing 10 mmol/L tri-butyl ammonium acetic acid; mobile phase B: methanol; gradient program: 0 %B (0 -0.5 min) - 25%B (7.5 min) -90%B (11-11.5 min) - 0 %B (11.6-15min) ; sample cycle time : 15 min. The mass spectrometric parameters were optimized automatically by flow injection analysis.

LCMS-8030 provides a high throughput quantitative analysis with the high chromatographic resolving power of UHPLC systems. In this study, we developed the quantification system for metabolites included in central pathway of energy metabolism with cycle time 15 min by ion-pare and reversed-phase chromatography coupled to LCMS-8030 with modification for improving its sensitivity.

Most metabolites in central pathway of energy metabolism show high hydrophilic property and have an acidic functional group. All compounds were detected as deprotonated molecules ([M-H] -) in ESI negative ion mode, and mass spectrometric parameters for MRM analysis such as monitoring ion and collision energy were optimized by automatic optimization procedure. In comparison of several brands of ODS column, we chose L-Column ODS in terms of separation of sugar phosphates.

As the results of optimization for chromatographic and mass spectrometric parameters, over 80 metabolites could be analyzed simultaneously in injection cycle time 15 min. The metabolites range from *m/z* 73 to *m/z* 866 and include ribitol as an internal standard. The analytical method we developed could also analyze non-volatile compounds such as nucleotides which could not analyzed by GCMS, and stability of retention time was satisfactory. We analyzed both of standard samples and cellular extracts to evaluate the qualitative capability such as limit of detection, dynamic range, carry over, and stability.

Yeast extract was analyzed as model samples to confirm applicability of developed method to biological samples. 75 components were detected successfully. Furthermore, we analyzed yeast extract with serial dilution to confirm linearity of metabolites quantity observed by this method. These results show that this method could detect change of a metabolite quantitatively.

In this study, we developed and evaluate comprehensive, ragged, and rapid quantitative system for metabolites in central pathway of energy metabolism. The result suggests that this system could be broadly applicable as a tool for widely targeted metabolomics.

PWe-024

13:30 – 14:40

Metabolic changes during aging complementary GC-EI-MS and GC-APCI-TOF-MS analysis of a short-lived mitochondrial knockdown of Caenorhabditis elegans

Jouji Seta[1], Verena Tellstroem[3], Aiko Barsch[3], Gabriele Zurek[3], Carsten Jaeger[2], Bernd Kammerer[2]

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Keywords:
GC/MS, GC-APCI, QTOF, metabolite, c. elegans

Novel aspects:
Metabolic profiling in Caenorhabditis elegans using GC-EI-MS and GC-APCI-TOF-MS

Abstract:
Aging is a highly complex biological process involving a multitude of alterations at the metabolic, cellular and organismal level. According to the free radical theory of aging, mitochondria are the major source of oxidative stress within the cell and a main cause of aging. Mitochondria may become increasingly dysfunctional with age and fundamental impacts on cellular energy metabolism may occur. The simple nematode worm *Caenorhabditis elegans* is a well-established model system for aging ; several mitochondrial mutations significantly reduce life span. However, metabolic alterations associated with life span reduction are largely unknown.

We compared whole-organism methanolic extracts of short-lived *C. elegans* depeleted in mitochondrial prohibition to those of non-treated *C. elegans* with normal life span. Non-targeted metabolite profiling of methoximated and trimethylsilylated compounds was performed with gas chromatography-mass spectrometry (GC-MS) with electron impact (EI) ionization. Peak areas were normalized to internal standard and sample dry weight, peak identification was achieved by database-assisted mass spectral/retention index matching (AMDIS, FiehnLib/NIST05) . In addition, gas chromatography coupled to high resolution TOF-MS with softer atmospheric ionization (APCI) was used to analyze the same methanolic extracts both for metabolic profiling and subsequent identification of known and unknown compounds. Molecular formulae were derived from the accurate mass and isotopic pattern data and searched in public databases (PubChem, KEGG) .

Initial profiling with GC-EI-MS yielded 212 reconstructed mass spectra present in at least 40 of 48 chromatograms. Of these 212 mass spectra, 134 could be assigned structures at a match factor of 800, while the rest remained unidentified. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) both allowed clear differentiation between metabolic phenotypes of short-lived and normal-lived worms. PLS-DA indicated that a set of 17 compounds was particularly relevant for phenotype differentiation ; however, five of these relevant metabolites could not be identified by database search.

In contrast to GC-EI-MS, GC-APCI-TOF-MS analysis readily yields pseudomolecular ions facilitating the identification of unknowns. Feature extraction from the GC-APCI-TOF-MS analyses for statistical analysis resulted in approx. 650 features/chromatogram. Differentiation using pattern recognition methods between RNAi-treated and non-treated animals was similarly possible based on the APCI data. Sum formula generation from accurate mass and isotopic pattern successfully confirmed identities of primary metabolites from GC-EI-MS analysis such as glycine or alanine. The identification of the unknown 5 phenotype related metabolites is currently ongoing.

PWe-025
11:10 – 12:20

Development of automated data processing tool for large scale MRM experiment in metabolomics study

Hiroshi Tsugawa[1], Mitsuhiro Kanazawa[2], Atsushi Ogiwara[2], Takeshi Bamba[1], Eiichiro Fukusaki[1]

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Keywords:
Metabolomcis, Data analysis, Multiple reaction monitoring

Novel aspects:
Development of an automated data processing tool to accurately and objectively identify the detected peaks by multiple reaction monitoring method

Abstract:
Multiple reaction monitoring (MRM) is a well-known mass spectrometric method to detect and quantify the target metabolites at high sensitivity and selectivity in complex samples. Triple quadrupole mass spectrometer capable of high-speed scanning has been increasingly developed to obtain a huge amount of compounds, and total number of transitions is more than 1000 in a single run. This large scale MRM method is frequently used for "widely targeted metabolomics " as a trend methodology to obtain a lot of metabolite information from 50 to 100 compounds.
MS instruments and their related assays have been remarkably improved, but data analysis tools are less developed accompany with large scale data sets. This is the reason why MRM measurement data are mostly evaluated by manual verifications. Manual verifications for MRM data are not only laborious tasks especially peak identification, but it causes more severely situations due to subjective and irreproducible approach.
To address this situation, we developed an automated data analysis tool for "widely targeted metabolomics ". It is easy to perform large scale MRM data especially for LC-MS/MS. Our challenges are algorithm developments to detect peaks without any subjects, and to accurately identify targeted compounds by LC-MS/MS. Existing peak detection algorithms are based on eluted shape of target compound. This tool utilizes retention time alignment among comparative samples and transitions along with the existing algorithm. As for accurate target compound identification, we constructed a reference library that has intensity ratio regarding multiple transitions for every target compound. Intensity ratios are referred to identify target compounds, and it differentiates target compounds that have same precursor and fragment *m/z*. Scores of the retention time similarity, peak shape similarity and correlation with the reference transition data are calculated, and total score is utilized to check whether the detected peak is the real target or not. In addition to the peak identification algorithm, our data analysis tool offers an organized data matrix from large amount of data sets for further statistical analysis directly.
We performed three experiments in order to demonstrate the main features of our tool. In this study, we utilized the ion-pair LC-MS/MS system which is used for sensitive analysis of central metabolites such as sugar phosphates and cofactors. The first experiment validated the accuracy of peak identification by analyzing a defined mixture of 72 standard compounds and the yeast extract spiked the mixture. The second experiment determined the quantitative performance by analyzing a defined mixture of 10 standard compounds measured at different concentrations. Finally, we validated the usefulness and versatility by using real biological samples. Our platform has been applied to perform widely targeted metabolomics of a lot of ion-pair LC-MS/MS chromatogram datasets of *Saccharomyces cerevisiae* cells sampled at different time points during the diauxic shift which is awell-known phenomenon in the yeast cell.

PWe-027
11:10 – 12:20

LC-MS/MS-based plasma metabonomics: finding potential biomarkers for lung cancer

Wei Yang[1], Yanhua Chen[1], Cong Xi[1], Ruiping Zhang[1], Yongmei Song[2], Jiuming He[1], Jinfa Bai[1], Qimin Zhan[2], Lvhua Wang[2], Nan Bi[2], Zeper Abliz*[1]

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Keywords:
Metabonomics ; LC-MS/MS ; Lung Cancer ; Plasma

Novel aspects:
In the study, the influence range and influence extent of the solvent effect has been studied in detail so that more accurate data could be got.

Abstract:

Introduction

Metabonomics is an important platform for understanding of the metabolites of integrated living systems and its dynamic responses to the changes caused by both endogenous and exogenous factors. Our laboratory has developed the method of metabonomics inprevious study. However, we found that re-dissolution solvents of dried plasma extracts lead to much different chromatographic behavior. In this research, the re-dissolution solvents have been optimized systematically for plasma metabonomics study.

The developed method of plasma metabonomics based on LC-MS/MS, multivariate statistics and metabolic correlation networks has been implemented to find biologically significant metabolite biomarkers in lung cancer. This strategy not only helps identify potential biomarkers for prognoses of early-stage disease, but also provides biochemical insights into changes in lung cancer.

Methods

232 lung cancer patients, including adenocarcinoma (n= 65) , quamous cell carcinoma (n=32) , small cell carcinoma (n=49) and blind samples of lung carcinoma (n=86) , and 156 healthy volunteers were recruited from the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences, Beijing, China. Proteins of plasma samples were removed with ice-cold acetonitrile. Then the supernatant was collected and completely evaporated to dryness in a SpeedVac concentrator (Thermo Savant) . The sample residues were dissolved in initial mobile phase and mixed by a vortex-mixer on ice. Then the samples were filtered with 96-well plate filter and injected with 96-well collection plate (Agilent, Captiva ND) . Rapid resolution liquid chromatography coupled to Q-TOFMS/MS system with ESI, were employed to detect the prepared plasma samples.

Raw LC-MS data files were converted into mzData format using Wiff to mzData utility (Applied Biosystems/MDS Sciex) and directly processed by open-source XCMS package under R statistical software (version 2.10.0) to carry out peak discrimination, filtering and alignment. The matrices were introduced into SIMCA-P software (Umetrics AB, Umeå, Sweden) for multivariate analysis.

Preliminary data

The samples that have been dried in a SpeedVac Concentrator were redissolved in different ratios of acetonitrile. It was manifested that the solvent effect could be presented when the solution is different from the initial mobile phase, even though there is only a tiny change. It was noting that the compounds eluted before 10 min could not be monitored when the samples were redissolved in 100 % acetonitrile. The larger deviation of the acetonitrile ratio between the solution and the initial mobile phase, the wider influence range and stronger influence degree of the solvent effect could occur. Now the influence range and influence extent of the solvent effect has been studied in detail.

The acquired LC-MS data of squamous carcinoma were imported into XCMS, yielding 1064 peaks of positive ions from 0 to 30 min of retention time. The resulting data was exported into SIMCA-P software for subsequent processing by multivariate data analysis. And 35 metabolites have been regarded as potential biomarkers. Until now, 15 potential biomarkers have been identified, including glucose, amino acids, carnitines and lysophosphatidylcholines. Next we will identify the potential biomarkers of adenocarcinoma and small cell lung cancer. And the potential biomarkers will be validated with the blind samples of lung carcinoma.

Acknowledgement

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PWe-028 Metabolomics and its application to high resolution phenotype analysis

13:30 – 14:40

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Keywords:
Metabolomis, Metabolic fingerprinting, Metabolic turnover analysis, PCA, PLS

Novel aspects:
Power of metabolome as a high resolution phenotype analysis is proved. Early developmental stage of vertebrate was first predicted by metablome. Strategic lifespan extension was performed using metabolome information.

Abstract:

Introduction

Metabolomics is applicable without genome sequence information. Therefore, Metabolomics would be powerful analytical tool for commercially available plant and microorganisms. Among the operation of Metabolomics, metabolic fingerprinting, in which metabolic profile is used as fingerprint for samples' performance prediction and classification, is thought to be a possible tactics. On this occasion, practical operation of Metabolomics for high resolution phenotype analysis would be presented.

Metabolome analysis of early developmental stage of experimental animal

Metabolome analysis of early developmental embryo was conducted to prove metabolome can express early developmental stage quantitatively. Time course sampling of early developmental embryo, which were prepared by artificial insemination, was performed . The obtained early developmental embryos were subjected metabolome analysis. Principal component analysis (PCA) revealed that 1 st component clearly indicated developmental stage of embryo. Partial least square projection on latent structure (PLS) was carried out using 'metabolome' as explanatory variable and 'time after fertilization' as responsible variable. PLS regression successfully indicated developmental stage using one embryo with only 30 minutes error. Similar experiment can be available for nematode (*C.elegans*) development.

Metabolomics based semi-rational prediction of yeast life span and its application to seeking of lifespan related mutants.

First, possibility of Metabolomics based prediction of "Life span ", which is highly integrated and complicated quantitative phenotype, was studied to find strong relationship between metabolome and lifespan by means of PCA. Orthogonal projection on latent structure (OPLS) based model can predict yeast life span using metabolome as fingerprint. Using information of principal component vector in PCA life span related metabolites were nominated and life span related genes were speculated.

Dynamic metabolome analysis by stable isotope dilution based time course sampling

Metabolic profiling usually imply a static analysis based on multiple snapshot of metabolome. In some case, dynamic information would be necessary. Metabolic flux analysis is one of the most important tactics for dynamic metabolic analysis. But the method is rather difficult for person who is not familiar with metabolic engineering. Time course sampling after labeling with stable isotopomer metabolites would be useful for apparent metabolic turn over analysis. Some preliminary experimental data are also discussed.

PWe-029
11:10 – 12:20

Implementation of a Data Independent MS/MS Acquisition Strategy for Metabolites Identification in a Metabolomics MS-based Workflow

Emmanuel Varesio, Gerard Hopfgartner

University of Geneva, Geneva, Switzerland

Keywords:
LC-MS/MS, Metabolites Identification, SWATH, High Resolution Mass Spectrometry, Human Urinary Metabolites

Novel aspects:
Alternative MS/MS acquisition strategy for metabolites identification in metabolomics MS-based workflows.

Abstract:
Mass spectrometry has emerged in metabolomic studies due to its high sensitivity and throughput, as well as its direct hyphenation to liquid chromatography when analyzing complex samples. High-resolution mass spectrometers are used in unsupervised LC-MS based assays to propose elemental formulae for the biomarker candidates. Following a search in metabolomics databases (e.g. KEGG, HMDB, PubChem) , MS/MS experiments are usually performed for structural investigations. MS/MS acquisition can be done either "on-the-fly " as an information-dependent acquisition (i.e.IDA) mode, which results in a biased information since the less abundant candidates are usually not submitted to collision-induced fragmentation, or as targeted experiments that require the re-analysis of the samples. We propose an alternative MS/MS acquisition strategy : the use of Sequential acquisition Windows of All Theoretical precursor ions (SWATH) approach or Global Precursor ions Scans (GPS) to offer unbiased information during metabolomics assays.
In this study, a set of compounds relevant as urinary metabolites was selected. Analytes structures were downloaded from the PubChem Compound database and molecular properties (*e.g.,* pKa, logD, logP) were calculated by using the ACD Labs software suite. The standard mixture (100 ng/ml) was first injected in reversed phase chromatography to characterize the analytes retention as well as the LC peak width to determine the optimal MS duty cycle. Typical LC peak width at the base was of 6 s that allows a maximum MS duty cycle of *ca.* 500ms. In IDA mode, the MS duty cycle could reach a maximum of 450 ms in order to get sufficient data point to define the LC peak accurately in TOF mode ; MS/MS spectra were acquired in dynamic background subtraction mode to improve spectra quality. Contrary to the IDA approach the SWATH or GPS acquisition mode is an alternative solution with several advantages in metabolites identification : i) some selectivity is put in the precursor ion selection (*i.e.*windows of 100u) , ii) potentially all the precursor ions undergo fragmentation since MS/MS spectra are acquired with a CE ramp, and iii) the precursor ion is present in the same spectrum as its fragments, which enables biomarkers quantification. These different strategies will be applied to identify metabolites in diluted or extracted urine samples using database search and elemental formulae assignment.

PWe-030
13:30 – 14:40

Alkyl chloroformate - mediated derivatization - extraction platform for parallel GC-MS and LC-MS based metabolomics

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Biology Centre, Czech Academy of Sciences

Keywords:
alkyl chloroformate-mediated derivatization - extraction, GC-MS, LC-MS, metabolomics

Novel aspects:
Alkyl chloroformate derivatization - extraction has been an efficient analytical platform complement to the current GC-MS & LC-MS based metabolomic analytical strategies

Abstract:
Comprehensive metabolomic analysis by GC-MS requires a preceding derivatization step relying mostly on the established (oximation-) silylation procedure [1]. However, metabolites are found mainly in aqueous environments with high concentrations of salts and polymeric components and usually are maintained in biochemical buffers while the silylation reaction must be performed under strictly anhydrous conditions. In this context, derivatization with an alkyl chloroformate (RCF) and/or the corresponding alcohol (ROH), and coupled with simultaneous liquid liquid extraction, has been an attractive option in multi-class mass spectrometric metabolite analysis. The RCF-ROH reaction proceeds smoothly with most protic groups in complex aqueous media under physiological conditions, even when the samples are treated/stored with most common biochemical buffers at molar concentration levels. Moreover, the typical arising carbamates-carbonate-ester derivatives are efficiently extractable into an immiscible organic phase and have moderate polarities. They are much easier prepared and considerably more stable than their silylated counterparts. Availability of the reagents with various (fluoro) alkyls, including those labeled with stable isotopes, make the RCF-mediated derivatization-extraction platform attractive in chiral GC-MS and parallel GC-MS & LC-MS based metabolomic analysis.
In this presentation, some issues important for the maturity of the RCF methodology will be briefly discussed, including :
(i) the effect of deproteination on the RCF-mediated derivatization-extraction [2],
(ii) the combined release of the disulfide-bound metabolites and its use in metabolomic analysis [3],
(iii) potential of fluoroalkyl chloroformate reagents including the chiral analysis [4],
(iv) the RCF labeling for the identification and analysis of metabolites and xenometabolites including the potential of the RCF reagents labeled with stable isotopes [5],
(v) matrix effects in LC-MS analysis [6],
(vi) application of the RCF-mediated derivatization - extraction platform in parallel GC-MS and LC-MS based insect metabolomics [7-9] leading to the discovery of a simple strategy which makes an intolerant insect organism freeze tolerant [10].

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PWe-031
11:10 – 12:20

Aberrant Metabolism and Its Response to Treatment in Esophageal Squamous Cell Carcinoma Revealed by Plasma Metabolomics Study

Jing Xu[1], Yanhua Chen[1], Ruiping Zhang[1], Yongmei Song[2], Nan Bi[2], Jiuming He[1], Jinfa Bai[1], Lvhua Wang[2], Qimin Zhan[2], Zeper Abliz[1]

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Keywords:
metabolomics ; esophageal carcinoma ; potential diagnostic biomarkers ; therapeutic biomarkers ; TCA cycle

Novel aspects:
Some potential diagnostic and therapeutic biomarkers by analyzing global changes in an individual's metabolic profile in response to pathophysiological stimuli or treatment intervention were discoverd.

Abstract:

Introduction

Metabolomics provides a powerful approach to discover diagnostic and therapeutic biomarkers by analyzing global changes in an individual's metabolic profile in response to pathophysiological stimuli or treatment intervention. At present, a plasma metabolomics study on esophageal squamous cell carcinoma (ESCC) and patients with treatment have been investigated by rapid resolution liquid chromatography -quadrupole time-of-flight mass spectrometry (RRLC-QTOFMS) in conjunction with multivariate statistics analysis (MVDA) . The metabolic profiles between pre-treatment patients and controls were performed to find the potential biomarkers closely associated with the disease. The metabolic profiles between pre- and post-treatment ESCC patients combined with the therapeutic evaluation was carried out to discover the major metabolites contributing to the discrimination which might be potential therapeutic biomarkers. The metabolite variations in post-treatment ESCC patients were further discussed and might be used to monitor treatment progress and presume outcome.

Methods

Pre-treatment samples : 44 males diagnosed with ESCC were recruited from the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences. Mid- and Post-treatment samples : Plasma samples were collected after the first course of 5-FU/CDDP based CRT (ESCC-M group) and the completion of CRT (ESCC-P group) from 32 ESCC male patients with different response to treatment (overall responders, OR group, n=21 ; non overall responders, non-OR group, n=11) . RRLC coupled with Q-TOF MS/MS system (QSTAR™ Elite) in electrospray ionization (ESI) positive and negative ion modes were used for metabolomics analysis after acetonitrile precipitation as sample preparation. The date treatment was processed by XCMS package, SIMCA-P software 12.0 (Umetrics AB, Umeå, Sweden) and SPSS software 17.0.

Preliminary data and Results

Metabolic perturbations under pathological conditions were investigated by metabolic profile between ESCC and NC. Major fifty-two metabolites contributing to the discrimination were screened and twenty-seven were identified as lipid metabolites (lysophosphatidylcholines (lysoPCs) , fatty acids) , lipid metabolism intermediates (carnitine and acylcarnitines) , amino acids, organic acids, sterol metabolite and monosaccharide. The abnormal levels of these metabolites indicated that the disturbed TCA cycles and the energy metabolism via b-oxidation occurred in ESCC patients. The AUC of the combined metabolites showed the good discrimination power. Furthermore, on the basis of these screened metabolites, the correlation network between them was constructed and a metabolic pathway related to ESCC was proposed in-depth.

To evaluate the physiological responses to treatment, OPLS-DA was performed to discriminate between the pre- and post-treatment metabolic profiles of ESCC patients. The metabolic alterations in ORs and non-ORs were also evaluated, respectively. Those significantly altered metabolites in ORs are more likely to be associated with the therapeutic effects. Thirteen discriminating variables between total pre- and post-treatment groups were identified. Eleven of these selected metabolites were accountable for the discrimination between pre- and post-treatment metabolic profiles in treatment ORs. Furthermore, after screening between two groups in treatment non-ORs, five metabolites were picked out and they might be the potential therapeutic biomarkers finally.

In addition, the variations of these five metabolites during pre-, mid- and post-treatment were researched. The result indicated that these five metabolites might closely relate with treatment effect and might be utlized to monitor treatment progress and presume outcome.

Acknowledgments

National Instrumentation Program (2011YQ170067)

PWe-032
13:30 – 14:40

Imaging mass spectrometry and structural analysis of lipids directly on tissue specimens using a high resolution MALDI-TOF-TOF tandem mass spectrometer

Shuichi Shimma[2], Ayumi Kubo[3], Takaya Satoh[3], Hisanao Hazama[1], Kunio Awazu[1], Michisato Toyoda[1]

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Keywords:
Imaging mass spectrometry, high resolution, tandem mass spectrometry

Novel aspects:
Direct tissue analysis and imagingmass spectrometry (IMS) was performed by using a high mass resolution tandem time-of-flight mass spectrometer

Abstract:

Introduction

Direct tissue analysis and imaging mass spectrometry (IMS) using a high mass resolution tandem time-of-flight (TOF) mass spectrometer is described. This system mainly consists of a spiral ion trajectory TOF mass spectrometer "SpiralTOF (STOF) " and an offset parabolic reflectron (RTOF) . Due to 17-m flight length in STOF, high mass resolution and high precursor selectivity are available. Elimination of metastable ions in STOF allowed observation of the product ions inherent to the high energy collision induced dissociation (HE-CID) process. By using this TOF-TOF instrument, the product ion spectrum of lipids provided detailed structural information of fatty acid residues. In this presentation, we will report advantages of high mass resolution IMS and structural analysis using HE-CID for lipid analysis.

Methods

This study used 6-week-old male C57BL/6J mice. The mice were anesthetized with isoflurane, sacrificed, and dissected. The brain block was immediately frozen in powdered dry ice to minimize degradation and kept at -80 degrees C. The tissue sections were sliced in 10 um-thick sections using a cryostat and thaw-mounted on a ITO slide glass sample plate (25 mm x 75 mm and thickness of 1.1 mm) . The samples were dehydrated in a vacuum chamber and the plate was mounted on a custom-built sample holder. The instrument was equipped with a 349 nm Nd : YLF laser for matrix-assisted laser desorption/ionization (MALDI) . In IMS experiment, the interval of data points was 80 um and numbers of laser irradiation in spot were 250 shots.

Preliminary Data

In both positive and negative ion modes, typical mass resolution of approximately 40,000 were obtained. Even in tissue analysis, the high mass resolution was sufficient to separate monoisotopic ions easily for the subsequent MS/MS experiment.

Positive ion detection mode provided a product ion peak of *m/z* 798, arising from the potassium ion adduct of phosphatidylcholine (PC) (34 : 1) . In product ion spectrum obtained using our system, several intense peaks were derived from the polar head group. Furthermore, a regular pattern of peaks was obtained above *m/z* 500. By detailed analysis of the high resolution mass spectrum, we found several doublet peaks which mass differed 0.1-0.2 u each other. For example, the [M+2] of *m/z* 866 and [M] of *m/z* 868 were observed in 0.18 u difference. By describing the mass image for each peak, former and latter ions are characteristically distributed on gray and white matters in mouse brain tissue section, respectively. The clear separation of IMS images is advantage of high resolution STOF that could not achieved by conventional reflectron type instrument.

Detail structural analysis showed product ions derived from dissociation of both *sn*-1 and *sn*-2 fatty acids. The CFR fragment ion peaks are clearly observed in product ion spectrum from *m/z* 798, which was expected as PC (34 : 1) . To validate this result, we confirmed the pattern of product ion spectra using a standard sample of PC (34 : 1) . In this presentation, we will discuss detailed structure of major lipid peaks observed in both positive and negative ion modes.

PWe-033
11:10 – 12:20

Observation of distributions of Strontium and Cesium stable isotopes in tissues sections of medaka by a new mass imaging system

Shinichiro Ikeda, Jun Aoki, Michisato Toyoda

Osaka University, Osaka, Japan

Keywords:
Imaging Mass Spectrometry

Novel aspects:
The distributions of strontium and cesium stable isotopes in tissue sections of small fish was obtained by imaging mass spectrometry.

Abstract:
Mechanism of biological absorption and accumulation of toxic substance is important matter and well studied. In particular the case of food, a information about distribution of the bioaccumulative toxins become significant from the aspect of safety for human health. Recently, the biological accumulation of radioactive substance became great concern due to the accident of nuclear power plant in Japan.

Conventionally, the biological distribution of radioactive substance is measured by autoradiography analysis. In the autoradiography, localization of decay emissions of radioactive substance in the measurement sample is imaged by an x-ray film. But this method takes up to about 1 week to get images and is impossible to identify the species of radioactive elements. Thus we developed new observation method to estimate the distribution of elements, for example strontium and cesium, in biological tissues by using newly developed imaging mass spectrometer (MULTUM-IMG 2). MULTUM-IMG 2 consisted of LDI ion source, multi-turn time-of-flight mass spectrometer (MULTUM) and a time and position sensitive detector. The sample surface is irradiated with UV laser at a beam diameter of about 0.5 mm. A spatial distribution of molecular on a sample surface is conserved optically through ionization and mass separation process and formed an image on the detection surface. The distribution information of mass of substances on the sample surface can be obtained by imaging mass spectrometry. Each elements and isotopes is identified by difference of mass, so measurement object is not limited to radioactive substance. We used non-radioactive isotopes, which is easy to handling, for this experiment. The biological activities of isotopes of same element is considered as no significant difference.

We chose medaka (*Oryzias latipes*) as measurement test subject and ^{88}Sr and ^{133}Cs as bioaccumulation elements. Medaka bred in 0.001 mol/l $SrCl_2$ and 0.05 mol/l CsI solution for two weeks absorbed these elements. The length of body is about 20 mm and the withers height is about 6 mm. For sample preparation, the frozen body was sliced into a thickness of 10 μm using a cryostat microtome and mounted onto a sample plate. The direction of slicing is from the cephalic region to the caudal region. The sample tissue of whole body was trimmed one-half from cephalic end fit to the sample plate of 10 mm in width. The sample surface was coated by a thin gold layer of about 10 nm for electrical conductivity, because electrostatic charge accumulation induced by laser irradiation and ionization deteriorate the image quality. Sample section was installed into the imaging mass spectrometer after acquisition of an optical microscope image. The view observed in one measurement is 500 μm diameter and we measured 50 times to observe the whole cephalic region.

Biological structure shown in the ion image was verified to be consistent with those in the optical microscope image. The resulting image showed that distribution profile was different in elements belonging to the same family, for example Na, K and Cs. Mass resolving power of $m/\Delta m$ 3450 were obtained for the main peak of ^{133}Cs at m/z = 132.9. Spatial resolution is enough to identify a biological structure of about 6 μm size. As the next step, this result is expected to be analyzed in detail with additional anatomical knowledge.

PWe-034 Investigation of fragmentation routes of steroid ethers via MIKES and B2*E-linked-scan techniques.

13:30 – 14:40

Heiko Bannick, Juergen Grotemeyer

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Keywords:
B 2 *E-linked-scan, MIKES

Novel aspects:
Fragmentation routes of steroid ethers have been investigated.

Abstract:
Introduction :

Metastable ions have been studied using MIKE- and B^2*E-linked-scan techniques to investigate the fragmentation of estradiol- and estriolethers appearing in the EI- or CI-mass spectra. The aim was to find out how a fragment is built : during a normal elimination process, following a rearrangement or during another consecutive process. Using this spectral data it is possible to draw a "fragmentation tree " were fragmentation routes are shown.

Methods :

In this work a ZAB 2 F build by Vaccuum Generators was used. Its sector fields are assembled in reversed Nier-Johnson geometry. The EI-ions were generated at 70 eV and ammonia was used as reagent gas in the CI-process.
To obtain the MIKE spectra a fragment ion was selected by the magnet and the E-field was scanned. In B^2*E modus the B- and E-fields were scanned at a constant ratio to point out the precursor ions.

Preliminary data :

The MIKE spectrum of the Estriol- 3 -benzylether radical cation shows the following eliminations : -18, -28, -36, -41, -55, -59, -91, -109, -116, -127 and -181 Da. Using MIKES and B^2*E-linked scans it is possible to check if these fragments are directly formed by elimination, rearrangements or in a consecutive elimination of two or more precursors.
An example for this is the elimination of 109 Da from Estriol- 3 -benzylether radical cation. This is an elimination of the ether group as a radical and the elimination of a water molecule. Following a consecutive reaction mechanism beginning with the loss of water, the MIKE spectrum of the $[M^{+\cdot}-18]$ should show an elimination of 91 Da which rather appears with quite low intensity. In addition the B^2*E-linked spectrum of the $[M^{+\cdot}-109]$ should show the $[M^{+\cdot}-18]$ as precursor, it does not. Instead it shows the $[M^{+}91]$.
Looking at the MIKE spectrum of the $[M^{+\cdot}-91]$ the loss of a water molecule is the most intensive elimination.
This indicates that first the ether group is eliminated and adjacent the water molecule.
Following this example the methyl-, ethyl-, propyl-, allyl-, and benzylethers of estradiol and estriol have been examined and the fragmentation routes have been determined.

PWe-035

11:10 – 12:20

Marked difference in fragmentation pathway between chemi-excitation and collision-induced excitation of CT-induced decomposition products of dioxetanes in negative-mode MALDI-TOF-MS/MS

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Keywords:

Dioxetane, CTID, MALDI-TOF-CID-MS/MS, Collision-induced-excitation, Electronically-chemi-excitation

Novel aspects:

We found that molecule-related ion underwent different types of fragmentation depending on the excitation node, i.e. chemi excitation or vibrational excitation.

Abstract:

A high-energy molecule, dioxetane, bearing an aromatic electron donor displays chemiluminescence by intramolecular charge-transfer (CT) -induced decomposition (CTID) mechanism, in which CT occurs from the electron donor to dioxetane O-O to produce excited species.

On the other hand, we have very recently reported that electron-transfer ionization takes place for dioxetanes bearing a hydroxyphenyl moiety substituted further with an aromatic ring that acts as an antenna to capture an electron from a matrix, such as poly（3-octylthiophene-2,5-diyl）, with low oxidation potential in negative-mode MALDI-TOF-MS.

Herein, we disclose that dioxetane **1** bearing a 3-hydroxyphenyl moiety substituted with benzothiazol-2-yl group undergoes intramolecular CT-induced decomposition to give keto ester **2** in the electronic excited state through the ionization in MS^1 mode described above, while authentic **2** gives a species in the vibrational excited state in MS^2 mode.

Dioxetanes **1** produced characteristic fragment ions depending on the substituent at the 5-position in MS^1 mode. Thus, dioxetane, 1-[4-(benzothiazol-2-yl)-3-hydroxyphenyl]-5-*tert*-butyl-4,4-dimethyl-2,6,7-trioxabicyclo [3.2.0] heptane **1a** producedfragment ions due to the elimination of 2-methyl-1-propene (56*u*) or pivalaldehyde (86*u*) (by α-cleavage of ketone moiety) from molecule-related ions. These fragment ions could not form directly from a dioxetane if it retained the four-membered peroxide ring framework. One strong candidate capable of causing such fragmentation is keto ester, dimethyl-3-oxobutyl-4-(benzothiazol-2-yl)-3-hydroxybenzenecarboxylate **2a** which is generated by the CT-induced decomposition of dioxetane **1a**. Thus, the ionization of authentic keto ester **2a** was investigated in MS^1 mode. The observed molecule-related ions from **2a** resembled those of dioxetane **1a**. However, the expected fragment ions could hardly be detected. These results in MS^1 spectrum strongly suggested that keto ester produced by the decomposition of high-energy dioxetane through ionization in MS would be energetically different from authentic keto ester **2a**. Thus, we performed further in MS^2 mode measurements focusing on deprotonated ionfor dioxetane **1a**. In the MS^2 spectrum of deprotonated ionfrom **1a** as the precursor ion, we observed the fragment ions generated by the loss of 56*u* or 86*u* from the precursor ion. On the other hand, by MS^2 measurement of the precursor ion $[M\text{-}H]^-$from **2a**, we observed the same product ions as in the case of **1a**. Next, we carried out MS^1 and MS^2 of dioxetanes **1b-d** which were **1a** analogs in place of *tert*-butyl group at the 5-position, bearing isopropyl, ethyl or methyl, and their related keto esters **2b-2d**. In contrast to dioxetane **1a** andketo ester **2a**,dioxetane **1b-d** and the corresponding keto ester **2b-d** exhibited marked difference in the fragmentation between MS^1 and MS^2 spectra. In the MS^1 spectrum, **1b-d** gave the fragment ions due to α-cleavage of ketone moiety, while in the MS^2 spectrum, both **1b-d** and **2b-d** underwent β-cleavage of an aromatic ester moiety to show fragment ions.

In conclusion, the present study showed that dioxetanes **1** underwent intramolecular CT-induced decomposition to afford keto esters **2** in the electronically excited stateinMS^1, while authentic keto esters **2** underwent vibrational excitation in MS^2. Keto esters **2** in the vibrational excited state caused α-cleavage of a ketone moiety and/or β-cleavage of an aromatic ester moiety in MS^2, depending on the alkyl ketone moiety. The features of fragmentation for a vibrationally excited molecule may be affected by the ease of bond cleavage that was caused by acquiring collisional energy.

PWe-036
13:30 – 14:40

Fragmentation of the [M + Na]+ and [M + Li]+ Ions of Multiply Benzyloxy-substituted Arenes under ESI/CID conditions

Dietmar Kuck[1], Sandra Heitkamp[1], Matthias C Letzel[1], Ishtiaq Ahmed[2], Karsten Krohn[2]

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Keywords:
Benzyl phenyl ethers, sodiated arenes, catechin derivatives, ESI-CID mass spectrometry, benzyl radicals

Novel aspects:
Fragmentation of $[M + Na]^+$ and $[M + Li]^+$ ions of di- and multiply benzyloxy-substituted arenes, structure-specific loss of two benzyl radicals, specific retro-Diels-Alder reaction of $[M + Na]^+$ ions.

Abstract:
The fragmentation of sodiated organic molecules formed under electrospray conditions has become a ubiquitous feature in mass spectrometry. Representing neither protonated nor radical cationic species, the gas-phase ion (organic) chemistry of such $[M + Na]^+$ ions is not well understood but a rich source of surprise. This holds also true for per-*O*-benzylated polyphenols derived from dimeric gallocatechins (prodelphinidins) studied recently.[1] Besides a strikingly specific retro-Diels-Alder reaction of the flavanol rings, pairwise loss of benzyl radicals (2 $C_7H_7^{\cdot}$, 182 u) was observed. To screen the scope of this unusual fragmentation behaviour, the ESI-CID mass spectra of a series of multiple benzyl ethers of lower di- and oligophenolic compounds were analysed with respect to the fragmentation of the $[M + Na]^+$ as well as $[M + Li]^+$ quasi-molecular ions.
The successive loss of two $C_7H_7^{\cdot}$ radicals from the $[M + Na]^+$ and $[M + Li]^+$ ions under CID has been found to be the predominating or even exclusive fragmentation channel of simple di- and tribenzyloxy-substituted aromatics, provided that the $C_6H_5CH_2O$ groups are oriented ortho (or para) to each other. Additional functionalities, such as CO_2H, CHO, CH_2OH or OH groups at the central phenol ring do not interfere in most cases. The MS^3 spectra of the $[M + Na - C_7H_7]^{+\cdot}$ ions and further MS^n experiments reveal that only the second loss of $C_7H_7^{\cdot}$ occurs with particular ease. Pyrogallol derivatives show high abundance ratios $r = \{ [M + Na - 2\ C_7H_7]^+ \} : \{ [M + Na - C_7H_7]^{+\cdot} \} > 1$ due to the presence of at least two *ortho*-benzyloxy groups, whereas phloroglucinol derivatives mostly give values $r << 1$.This also holds true for higher analogues, such as hydroxychalcone and (gallo) catechin derivatives bearing up to six benzyloxy groups. It appears that twofold loss of two $C_7H_7^{\cdot}$ radicals becomes more predominant with increasing size of the $[M + Na]^+$ ions and that it is due to the favourable formation of *ortho*-quinoid structures in the fragment adduct ions.

1. K. Krohn, I. Ahmed, M. John, M. C. Letzel, D. Kuck, *Eur. J. Org. Chem.* **2010**, 2544-2554.

PWe-037
11:10 – 12:20

A functional group substitution effect on the free radical initiated peptide sequencing (FRIPS) method

Han Bin Oh[1], Jihye Lee[1], Hyeyeon Park[1], Kun Cho[1,2], Bongjin Moon[1]

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Keywords:
FRIPS, Peptide fragmentation, Radical, functional group substitution

Novel aspects:
When a new functional group is introduced to the o-TEMPO-Bz-NHS, new radical-induced peptide fragmentation patterns were shown.

Abstract:
Last decade, we have witnessed the development of various radical-based peptide sequencing methods. Representative examples include electron capture dissociation, electron transfer dissociation, photodissociation of photolabile group, UV photodissociation, collisional homolytic cleavage of radical precursor, and so on. In particular, we have paid attention to the collisional homolytic cleavage of radical precursor molecule, o-TEMPO-Bz-NHS. When collisional activation is applied to o-TEMPO-Bz-peptides, facile homolytic cleavage occurs between the benzyl carbon and the oxygen of TEMPO moiety, thus producing a benzyl radical peptide cations. Upon the additional collisional activation on these benzyl radical peptide cations, this localized radical site further induces peptide backbone fragmentations. These processes are refered to as 'free radical initiated peptide sequencing (FRIPS) '. In the current study, we added a functional group to the benzene ring in order to shed light on how a functional group substitution affects the radical induced peptide sequencing. The added functional group included NO_2, Br, OMe, and CN. Interestingly, the substituted o-TEMPO-benzyl group affected the FRIPS process, yielding MS^3 spectra with different fragmentation patterns. The precursor survival yields were also obtained as a function of the applied collision energy in order to shed light on the mechanistic aspect of the substitution effect on the FRIPS process. In the conference, the details of the observed mass spectra will be presented.

PWe-038 Evaluation of peptide fractionation strategies used in proteome analysis

13:30 – 14:40

Chih-Wei Chiu, Chun-Lun Chang, Sung-Fang Chen*

National Taiwan Normal University, Taipei, Taiwan

Keywords:

Two-dimensional HPLC, Peptide fractionation

Novel aspects:

Peptide fractionation using SCX, HILIC, alkaline-RP and s-IEF were evaluated. The salt content of a sample lowers separation efficiency of SCX. Combining complementary separation schemes can increase protein sequence coverage.

Abstract:

Peptide fractionation is extremely important for the comprehensive analysis of complex protein mixtures. Although a few in which the relative separation efficiencies of 2 D methodologies using complex biological samples are compared, a systematic evaluation was conducted in this study. Four different fractionation methods, including SCX, HILIC, alkaline-RP and solution IEF prior to LC-MS/MS analysis were compared. SCX x RP-LC, using desalted samples, permitted the greatest number of proteins to be identified (96.54 % of the total proteins were identified) . The result was followed closely by HILIC x RP-LC and alkaline-RP x RP-LC. It is noteworthy that, when SCX x RP-LC was used after desalting the sample, significantly more proteins were identified, compared with the non-desalted sample (1990 and 1375,respectively) . We also found that the use of a combination of analytical methods resulted in a dramatic increase in the number of unique peptides that were identified, compared with only a small increase in protein levels. The increased number of distinct peptides that can be identied is especially beneficial, not only for unequivocally identifying proteins but also for proteomic studies involving post-translational modifications and peptide-based quantication approaches using stable isotope labeling. The identication and quantication of more peptides per protein provide valuable information that improves both the quantication of, and confidence of protein identification.

PWe-039

11:10 – 12:20

Simultaneous analysis of 8-oxo-7, 8-dihydroguanine and 8-oxo-7, 8-dihydro-2'-deoxyguanosine in plasma by liquid chromatography-tandem mass spectrometry with automated solid-phase extraction

Yi-Hung Tsai, Mu-Rong Chao

Department of Occupational Safety and Health, Chung Shan Medical University, Taichung 402, Taiwan

Keywords:
oxidative stress, plasma, on-line SPE

Novel aspects:
Simultaneous analysis of 8-oxo-7, 8-dihydroguanine and 8-oxo-7, 8-dihydro-2'-deoxyguanosine in plasma by liquid chromatography-tandem mass spectrometry with automated solid-phase extraction

Abstract:
Oxidatively damaged DNA has been associated with the development of aging, cancer and some degenerative diseases. 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG, so called 8-OHdG) is the most abundant DNA lesion in cellular DNA, and its repair products in urine, namely modified base 8-oxo-7,8-dihydroguanine (8-oxoGua) and modified nucleoside (8-oxodG), have been widely studied and used as the biomarkers of oxidative stress. In this study, we developed an isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) with on-line solid-phase extraction (on-line SPE) for rapid and simultaneous analysis of 8-oxoGua and 8-oxodG in plasma.

Two clean-up procedures (i.e., protein precipitation and manual SPE) were used prior to on-line SPE LC-MS/MS analysis. An aliquot of prepared sample was then directly injected into the on-line SPE LC-MS/MS. The on-line SPE consisted of a switching value (two-position microelectric actuator; Valco) and a C18 trap column (75 × 2.1 mm i.d., 5 μm; Inertsil, ODS-3). The switching value function was controlled using PE-SCIEX control software (Analyst; Applied Biosystems). After automatic sample cleanup, the sample was automatically transferred onto a C18 column (150 × 2.1 mm i.d., 5 μm; Inertsil, ODS-3) using a Agilent 1100 series HPLC system interfaced with a PE Sciex API 3000 triple quadrupole mass spectrometer with electrospray ion source (ESI). The mobile phase comprised of various proportions of methanol and ammonium acetate solution. The samples were analyzed in the positive ion multiple reaction monitoring (MRM) mode. For all of samples, the $[M+H]^+$ ion was selected by the first mass filter. After collisional activation, two fragment ions were selected: the most abundant fragment ion was used for quantification (quantifier ion), and the second most abundant ion was used for qualification (qualifier ion). Optimal multiple reaction monitoring conditions were obtained for four channels to different period: 8-oxoGua (m/z 168 → 140 and 168 → 112), [$^{15}N_5$]-8-oxoGua (m/z 173 → 145 and 173 → 117), 8-oxodG (m/z 284 → 168 and 284 → 140), and [$^{15}N_5$]-8-oxodG (m/z 289 → 173 and 289 → 145). The dwell times per channel were set at 100 ms for both the analytes and internal standards.

With the use of isotopic internal standards and on-line SPE, the detection limits of 8-oxoGua and 8-oxodG were found to be 17.2 and 5.1 fmol, respectively. This newly developed method was further applied to determine the plasma levels of 8-oxoGua and 8-oxodG in 20 healthy adults. The results showed that the background plasma levels of 8-oxoGua and 8-oxodG were 0.076~0.305 ng/ml and 0.07~0.023 ng/ml, being far lower than those in urine (14~33 ng/ml for 8-oxoGua and 5~13 ng/ml for 8-oxodG). Furthermore, significant correlations were observed between plasma and urine for both 8-oxoGua and 8-oxodG concentrations, giving the possibility of using other body fluids in addition to urine specimen for assessing the whole-body burden of oxidative stress.

PWe-041

11:10 – 12:20

Quantitative analysis of nine N-nitrosamines in human urine by isotope-dilution liquid chromatography- tandem mass spectrometry with on-line solid-phase extraction

Ming-Yen Hsieh, Chiung-Wen Hu

Department of Public Health, Chung Shan Medical University, Taichung, Taiwan

Keywords:

N-nitrosamines, urine, on-line SPE, LC-MS/MS

Novel aspects:

Quantitative analysis of nine N-nitrosamines in human urine by isotope-dilution liquid chromatography- tandem mass spectrometry with on-line solid-phase extraction

Abstract:

N-nitrosamines are potentially carcinogenic, mutagenic and teratogenic compounds for animals and humans that are often found in food, drinking water and environment. In addition to exogenous exposure, *N*-nitrosamines can also be formed endogenously inside the body from its precursors, nitrate or nitrite and secondary or tertiary amines. In this study, we developed an isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) with on-line solid-phase extraction (on-line SPE) for a quantitative analysis of nine *N*-nitrosamines in human urine, namely *N*-nitrosodimethylamine (NDMA) , *N*-nitrosomethylethylamine (NMEA) , *N*-nitrosopyrrolidine (NPyr) , *N*-nitrosodiethylamine (NDEA) , *N*-nitrosopiperidine (NPip) , *N*-nitrosomorpholine (NMor) , *N*-nitrosodi-n-propylamine (NDPA) , *N*-nitrosodi-n-buthylamine (NDBA) and *N*-nitrosodiphenylamine (NDPhA) .

A 24 ml of crude urine added with isotopic internal standards, then purified by activated carbon and concentrated to 0.1 ml under a high purity nitrogen stream. A 10 μl aliquot of prepared sample was then directly injected into the on-line SPE LC-MS/MS. Automatic sample cleanup (on-line SPE) was obtained by using a switching value (two-position microelectric actuator ; Valco) and a C18 trap column (75 × 2.1 mm i.d., 5 μm, ODS-3, Inertsil) that was controlled using PE-SCIEX control software (Analyst ; Applied Biosystems) . After automatic sample cleanup, the sample was automatically transferred onto a C18 column (150 × 2.1 mm i.d., 5 μm, ODS-3, Inertsil) using a Agilent 1100 series HPLC system interfaced with a PE Sciex API 3000 triple quadrupole mass spectrometer with electrospray ion source (ESI) or atmospheric pressure chemical ionization (APCI) . For a satisfactory sensitivity or selectivity, the quantifications of NDMA, NMEA, NPyr and NMor were performed in the positive-ion APCI mode, while the quantifications of NDEA, NPip, NDPA, NDBA and NDPhA were performed in the positive-ion ESI mode. For all of the samples, the $[M+H]^+$ ion was selected by the first mass filter. After collisional activation, two fragment ions were selected : the most abundant fragment ion was used for quantification (quantifier ion) , and the second most abundant ion was used for qualification (qualifier ion) . Optimal multiple reaction monitoring conditions were obtained for 36 channels : NDMA (m/z 75→43 and 75→58) , NDMA-d_6 (m/z 81→46) ; NMEA (m/z 89→61 and 89→43) , NMEA-d_3 (m/z 92→64) ; NPyr (m/z 101→55 and 101→41) , NPyr-d_8 (m/z 109→62) ; NDEA (m/z 103→75 and 103→47) , NDEA-d_{10} (m/z 113→81) ; NPip (m/z 115→69 and 115→41) , NPip-d_{10} (m/z 125→78) ; NMor (m/z 117→87 and 117→73) , NMor-d_8 (m/z 125→95) ; NDPA (m/z 131→89 and 131→43) , NDPA-d_{14} (m/z 145→97) ; NDBA (m/z 159→103 and 159→57) ; NDPhA (m/z 199→169 and 199→66) , NDPhA-d_6 (m/z 205→175) . The dwell times per channel were set at 50 ms for all the analytes and internal standards.

With the use of isotopic internal standards and on-line SPE, the detection limits of NDMA, NMEA, NPyr, NMor were 23.9, 6.2, 15.5, and 0.57 pg in APCI mode, while NDEA, NPip, NDPA, NDBA and NDPhA were 9.3, 1.7, 1.0, 0.34 and 0.14 pg in ESI mode, respectively. This method was further applied to measure the urinary concentrations of nine *N*-nitrosamines in nonsmokers. The results showed that among nine *N*-nitrosamines only NDMA (˜0.34 ng/ml) , NPyr (˜1.35 ng/ml) , NDBA (˜0.015 ng/ml) and NDPhA (0.025 ng/ml) were detectable while the others were under LODs of our method. Overall, the present method would be useful to assess the human exposure to nitrosamines.

PWe-042

13:30 – 14:40

Ultra-Performance Liquid Chromatography/Mass Spectrometric Method for the determination of venlafaxine and its metabolite in plasma: Application to pharmacokinetic study

Sunil Kumar Dubey[1], Hemanth Jangala[1], R N Saha[1], S Pasha[2]

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Keywords:
Venlafaxine ; O-desmethylvenlafaxine ; metabolite ; UPLC-MS/ESI

Novel aspects:
First (UPLC-MS/ESI) method for simultaneous determination of venlafaxine (VEN) and its metabolites in rat plasma has been developed and validated.Mass Spectrometric conditions were optimised in a unique way.

Abstract:
A new Ultra-performance liquid chromatography-electrospray ionization mass spectrometry (UPLC-MS/ESI) method for simultaneous determination of venlafaxine (VEN) and its metabolite O-desmethylvenlafaxine (ODV) , in rat plasma has been developed and validated using Venlafaxine d 6 as the internal standard. The compounds and internal standard were extracted from plasma by solid phase extraction. The UPLC separation of the analytes was performed on ACQUITY UPLC ® BEH Shield RP18 (1.7µm, 100mm×2.1 mm) column, using isocratic elution with mobile phase constituted of water (containing 2 mmol/l ammonium acetate) : acetonitrile (20 : 80, v/v) at a flow-rate of 0.3 ml/min. All of the analytes were eluted within 1.5 min. The compounds were ionized in the electrospray ionization (ESI) ion source of the mass spectrometer, operating in multiple reaction monitoring (MRM) and positive ion mode. The precursor to product ion transitions monitored for VEN, ODV and IS were m/z 278.3→121.08, 264.2→107.1 and 284.4→121.0, respectively. Calibration curves in spiked plasma were linear from 10-2000 ng/ml for VEN and ODV, with coefficients of determination above 0.992 for both. The extraction recoveries for both the analytes were above 76%. The limits of detection were 2.66ng/ml for VEN and 2.78 ng/ml for ODV. The method was fully validated for its sensitivity, accuracy, precision, linearity, recovery, matrix effect, and stability studies. The developed method was used for the pharmacokinetic study of VEN in rats, carried out by administering a single dose (120 mg/kg) of VEN by oral route. The concentration of VEN and ODV, in plasma was estimated at different time points upto 24 hr post dosing. To 300µl plasma samples, 200µl mobile phase was added and the drug was eluted by methanol by using solid phase extraction. The eluates were evaporated to dryness at 40^0C under N_2 gas. Residues were then reconstituted in 300 µL of mobile phase. Seven microliters of the solution were injected for analysis through the auto-injector. Various pharmacokinetic parameters were determined by non compartmental technique using Winnolin. The AUC and C_{max} of VEN, was found to be 2.92 µg.hr/ml and 1.48 µg/ml and those of ODV were 2.33 µg.hr/ml and 0.47 µg/ml. Cl/F and V_d/F of VEN are 41.03 L/hr/kg and 185.58 L/kg respectively ; $t_{1/2}$ of VEN is 3.13 hr and 2.59 hr for ODV. Elimination rate constant, K_e for VEN and ODV are 0.221 hr^{-1} and 0.267hr^{-1}. The elimination half life of both VEN and ODV are found to be same. As T_{max} for VEN was found to be 0.5 hr and that of ODV was 1.0 hr, oral absorption of VEN and conversion to ODV both can be considered as fast. Based on C_{max} and AUC values it is evident that exposure to ODV was less than VEN, this might be due to conversion of more VEN into other metabolites other than ODV or more formed ODV is metabolized further. The method is accurate, sensitive and reliable for the pharmacokinetic study and therapeutic drug monitoring (TDM) of venlafaxine.

PWe-043

11:10 – 12:20

Quantitative evaluation of synthase and hemisynthase activity of glucosamine-6P synthase by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Vincent Guerineau, David Touboul, Florence Wieczorek, Marie-Ange Badet-Denisot, Bernard Badet, Alain Brunelle, Philippe Durand

ICSN CNRS, Gif sur Yvette, France

Keywords:
Quantification, MALDI-TOF, matrix, 9-aminoacridine, glucosamine-6P

Novel aspects:
Alternative analytical tool for the determination of enzymatic kinetic parameters with minimal sample preparation, rapid analysis, high specificity and high sensitivity

Abstract:

Introduction

Glucosamine-6-phosphate synthase (Glms, Gfa or Gfat) is a key in the enzyme metabolic pathway of amino sugar-containing macromolecules biosynthesis. Main function of Glms is the catalytic conversion of D-glucosamine-6P (GlcN-6P) and L-glutamate (Glu) into D-fructose-6P (Fru-6P) and L-glutamine (Gln), respectively. As Glms plays a crucial role in glucose homeostasis regulation and toxicity through desensitization of glucose transport system to insulin, it was considered as a possible molecular target for the treatment of type II diabetes. Enzyme assays for Glms have been already described for either synthase (Fru-6P converted into Glu-6P) or hemisynthase (Gln into Glu) activities using various analytical chemistry methods, such as fluorimetry, UV spectroscopy, colorimetry or radiometry. Most of these methods showed false-responses due to interferences and can be time- and cost-consuming. In order to overcome these limitations, MALDI was used for the detection and quantification of the two substrates and the two products in one shot.

Experimental method

Experiments were performed using a MALDI-TOF (Voyager DE-STR, ABSciex). Prior to analysis, the four analytes were acetylated using anhydrous acetic (10%). This derivation step was introduced due to the encountered difficulty when synthesizing ^{13}C-GlcN-6P internal standard. Buffer solution was optimized and MALDI matrices, *i.e.* trihydroxyacetophenone (THAP) and 9-aminoacridine (9-AA), were compared in terms of robustness and sensitivity.By fixing the concentration of either GlcN-6P or Glu, Michaelis constants K_m for synthase and hemisynthase activities were determined.

Results

In order to avoid signal extinction, TRIS (20 mM, pH 7.2) was chosen as the best buffer solution. THAP matrix appeared not to be suited for reproducible data acquisition because of non-homogeneous crystallization. Moreover, overlapping between isotopic distributions of $[THAP+Na]^+$ and $[N\text{-acetyl-4,4-d}_2\text{-glutamate} +H]^+$ ions makes impossible to monitor the enzyme activity. In the negative ion mode, an optimized sample preparation with 9-AA appeared to be more robust and reproducible. This last was chosen for the following experiments.

Determination of Fru-6P and Gln Michaelis constants for synthase and hemisynthase activities was performed by combining the results of two experiments. One consists in fixing the concentration of Glu and the second one in fixing the concentration of GlcN-6P. K_m (F6P) and K_m (Gln) were found to be around 400 and 150 µM, respectively. V_{max} was estimated at 15 µM.mL^{-1}. These values are in excellent agreement with those already published in the literature. MALDI can thus be considered as an alternative analytical tool for the determination of enzymatic kinetic parameters.

PWe-044
13:30 – 14:40

Optimization of ESI method for the LC-MS analysis of phosphorylated peptide

Issey Osaka, Mitsuo Takayama

Yokohama City University

Keywords:
ESI, ion yield, phosphorylated peptide

Novel aspects:
The influence of amino acids and phosphorylation on the ion yields of peptide and phosphorylated peptide has been evaluated to determine the optimized conditions for ESI-MS.

Abstract:
Peptide-mass fingerprinting (PMF) and amino acid sequencing using LC-MS have become common approaches for the characterization of proteins and determination of post-translational modifications. Although protein and peptide analysis using ESI-MS, which is often coupled to LC, has been performed in positive-ion mode because of its superior ion yields compared to the negative-ion mode, the use of both positive- and negative-ion mode provides useful information in protein identification and in the screening for phosphorylated-site sequences in protein digest. If all the peptide peaks of protein digest could be detected in mass spectra, accurate identification of protein would be achieved. However, the molecular ion yields of peptides and phosphorylated peptides are strongly dependent on their physicochemical properties, which originate from the individual nature of the side chains of the constituent amino acids. In order to understand the ion yields in ESI method, here we use a constitutional form where we have divided the total ionization processes J_i into ionization efficiency of analyte molecules I and the rate of desorption or vaporization of molecules J_v as follows :

$$J_i = IJ_v \ldots (1)$$

The efficiency I can be related to thermochemical quantities such as proton affinity, gas-phase basicity, ionization energy or electron affinity of a given analyte. The quantity J_v represents the ability of analyte molecules to desorb or vaporize from liquid- to gas-phase in ESI.

Amino acids have a wide variety of physicochemical properties such as acidity, basicity, hydrophobicity and hydrophilicity originated from the characteristics of the amino acid side chain. It has been reported that the presence of Arg, aliphatic and aromatic amino acid residues enhance the ion yields of peptide in MALDI-MS and FAB. However, the effect originated from Arg residue and phosphorylation on the ion yields was not evaluated in ESI. Therefore, the relationship between the ion yields and physicochemical properties remains unclear for LC-MS experiment used ESI, although LC-MS is recognized as an indispensable analytical tool for proteomics. In this study, we investigated factors governing the molecular ion yields of peptide in ESI, in order to optimize the ESI method for LC-MS analysis of phosphorylated peptide. The model peptides such as threonine (T) -cluster and ACTH-based series were used for the evaluation of ion yields.

An enhancement effect of an Arg residue on the ion yields of $[M+H]^+$ in positive-ion MALDI-MS was reported previously. In the positive-ion ESI-MS mass spectra of T7 (TTTTTTT), RT6 (RTTTTTT), ACTH22-39 (VYPNGAEDESAEAFPLEF) and [Arg^{22}] -ACTH22-39 (RYPNGAEDESAEAFPLEF), however, such enhancement effect of Arg residue was not observed. It was found that the number of basic site was no significant factor in ESI MS.

An enhancement effect of Phe residue on the ion yield of both $[M+H]^+$ and $[M-H]^-$ was observed by comparing Phe containing peptide RT5F (RTTTTTF), FT5R (FTTTTTR), ACTH18-35 (RPVKVYPNGAEDESAEAF) and [Arg^{36}] -ACTH18-36 (RPVKVYPNGAEDESAEAFR). It is likely that the aromatic group of the Phe residue is advantageous for the process of desorption from liquid-phase of charged droplet to gas-phase, because of its high surface activity. It is understood via the term of the rate of desorption of analyte molecule, J_v in equation 1. The ion yield of [Arg^{36}] -ACTH18-36 was poor, although the proton affinity of Arg residue is the highest. This may be due to the hydrophilic property of Arg in both N- and C-terminal of the peptide. Furthermore, the effects of phosphorylation and the variety of charged state distributions with sequence of peptide and additives of sample solution, such as acetate or ammonium acetate, on the ion yields have been evaluated quantitatively. The optimized conditions for the ESI-MS of peptides and phosphorylated peptide would be presented.

PWe-045
11:10 – 12:20

Amino acid analysis by LC/MS with LC columns designed for hydrophilic compounds

Hiroko Arai[1], Tsuneaki Kaneko[1], Taketoshi Kanda[1], Yanli Guo[2], Osamu Shirota[2]

[1]Shiseido, [2]Shiseido China

Keywords:
Amino acid, LC-MS, hydrophilic interaction chromatography, reversed-phase chromatography, ion-exchnage chromatography

Novel aspects:
Free amino acids were analyzed with LC-MS, by using LC columns specially designed for hydrophilic compounds.

Abstract:
Free amino acids, being polar and electrically charged, are generally considered unsuitable to common high-performance liquid chromatography (HPLC) with reversed-phase separation and UV detection. Many chemical derivatization techniques have been developed for them to date. This paper will describe simple methods to analyze free amino acids with liquid chromatography (LC) -mass spectrometry (MS) , using several unique LC columns designed for LC-MS of hydrophilic compounds. These are 1) CAPCELL PAK C18 AQ, or a column designed for 100% aqueous mobile phase, 2) CAPCELL PAK CR, or a column containing both strong cation exchanger and reversed phase, and 3) PC HILIC, or a column of hydrophilic interaction chromatography (to be referred as AQ, CR, and PC HILIC, respectively) . Separation characteristics and detection sensitivity of eighteen standard amino acids were studied by using LC-MS with the three columns.

AQ could retain aliphatic and aromatic amino acids adequately, while it showed small retention for acidic and basic ones. PC HILIC, on the contrary, showed a relatively large retention for acidic amino acids, and small retention for aliphatic and aromatic ones. Retention of basic amino acids on CR was larger than those on other two columns, and CR also could retain aliphatic and aromatic amino acids even under a mobile phase with an increased organic content. Although any single column did not show a complete separation of eighteen amino acids, LC-MS with these three columns seems simple and useful if one tries to quickly analyze a particular type of amino acids.

Poster Session

Wedensday, 19th September — Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 24: Regulated Bioanalysis

PWe-046 Accelerate Method Development using Fast Screening of Mobile Phases Additives and Solvents for Optimum Sensitivity in LC-MS

13:30 – 14:40

Mikael LEVI, Stephane MOREAU
Shimadzu France, Noisiel, France

Keywords:
LC-MS, sensitivity, optimization, mobile phase

Novel aspects:
First proposed methodology for rapid screening of mobile phase reagents for maximum ionization efficiency in LC-MS.

Abstract:
It is now well-known that mobile phase components (*i.e.* additives and solvents) play a major role in ionization efficiency. Laboratory facing challenges in fast method development and high sensitivity are often condemned to use generic mobile phases and to invest in expensive high-end mass spectrometers. Furthermore, recent developments in HPLC columns allow more flexibility in the use of acidic or basic additives as well as viscous solvents .To the best of our knowledge, no rapid and systematic methodology are proposed to quickly optimize HPLC mobile phase recipe from a MS sensitivity point of view.
Model compounds representing a wide panel of chemical classes were dissolved in several mobile phase mixtures. Compounds were chosen with different chemical moieties and hydrophobicity. They were also chosen in order to have both compounds ionized in positive or negative mode and some of them could only be ionized forming salt adducts. Mobile phase mixtures were elaborated using a rational combination of solvents with water and several additives including organic acids, bases and salts. These mixtures were then injected using flow injection analysis and a dummy mobile phase carrier.
Flow injection parameters (*e.g.* sample volume, flow rate, autosampler parameters) were optimized in order to have an unbiased overview of the mobile phase component impact on ionization. Conditions were also optimized to reduce the peak intensity standard deviation, leading to statistically significant differences observed in responses.
The results showed that unexpected or unintuitive mobile phase compositions lead to enhanced ionization efficiencies. This screening permits to choose mobile phase reagents in less than ten minutes.
While single component analysis allows rapid and clear decisions, in the case of multiclass component analysis (*e.g.* environmental analysis) , a methodology is proposed to choose the best compromise.
Results also show that the instrumentation is important to be able to quickly perform this screening. This includes autosampler performances and mass spectrometer acquisition speed.

PWe-047
11:10 – 12:20

Effective Strategies for Phospholipid Removal using Supported Liquid Extraction (SLE) with LC-MS/MS Analysis

Gavin J Jones, Lee Williams, Rhys Jones, Maiko Kaneko, Steve Jordan

Biotage, Cardiff, UK

Keywords:
Sample Preparation, Mass Spectrometry, ion suppression

Novel aspects:
Evaluation of phospholipid content using supported liquid extraction with a comprehensive array of extraction solvent and pH sample pre-treatment combinations.

Abstract:
Endogenous phospholipids in biological fluids present a major problem in LC-MS/MS analysis. Due to their strong retention characteristics in reversed phase chromatography phospholipids tend not to elute as discrete peaks and are often very difficult to separate from analytes of interest. Traditional liquid-liquid extraction (LLE) provides very clean extracts. Supported liquid extraction (SLE) is analogous to liquid-liquid extraction, however, as a solid supported technique it provides subtle differences in extract cleanliness. This poster compares phospholipid removal from biological fluids using a wide variety of solvent combinations, pH control and polar extraction solvents on supported liquid extraction plates.

Phospholipid removal was evaluated using 100 µL human plasma pre-treated with : 2 % HCl, 1 % formic acid (aq) , 0.1% formic acid (aq) , 100mM NH_4OAc, water and 0.5M ammonium hydroxide to cover the pH range from 1.5-10.5. The use of ion pair reagents was also investigated. Extraction was performed using various water immiscible extraction solvents eitheralone or in combination (1 mL) : 98 : 2 hexane : 3-methylbutanol, heptane, MTBE,DCM, 98 : 2, 95 : 5, 90 : 10 (v/v) DCM/IPA, butanol, chlorobutane and EtOAc. Extracts were evaporated to dryness and reconstituted in mobile phase. Samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. MRM transitions monitoring the phospholipid product ion at 184 Da were acquired using electrospray ionization.

Phospholipid ions were separated into lyso-phospholipids (494-524 Da) and larger molecular weight phospholipids (>701-811 Da) for comparison. Protein precipitated plasma samples were used as a benchmark to evaluate the degree of phospholipid removal under the various extraction conditions. Extraction solvents were selected to give varying solvent characteristics. 98 : 2 hexane/ 3-methylbutanol, heptane, MTBE and DCM generally resulted in low levels of larger molecular weight phospholipids and lyso PLs in the final extracts at all pH conditions. More polar extraction solvents such as EtOAc exhibited low levels of lyso PLs but contained the larger molecular weight PLs in the extract. Increasing percentages of IPA in DCM resulted in increased phospholipid content in the extracts. Where the use of increasingly polar extraction solvents was required, reductions in load and extraction volumes resulted in decreased PL content in the final extracts. The use of organic solvents in the load step to help disrupt protein binding is possible, however, careful selection of extract solvent was required. For example, using IPA at up to 25 % with pre-treated plasma resulted in low levels of PL in the extract. Substituting IPA with ACN resulted in higher PL levels, which nevertheless was substantially lower than the corresponding protein crash data.

PWe-048
13:30 – 14:40

Simultaneous determination seven hydroxyacids by capillary electrophoresis and liquid chromatography mass spectrometry

Yen-Ling Chen, Pei-Yu Liu

Kaohsiung Medical University, Kaohsiung , Taiwan

Keywords:
Capillary electrophoresis/LC-MS/ Hydroxy acids/Cosmetics

Novel aspects:
A simpel and rapid analysis method for simultaneous determination seven HAs

Abstract:
A simple cyclodextrin modified capillary electrophoresis (CDCE) method and liquid chromatography mass spectrometry (LC-MS) have been developed for analyzing seven hydroxyl acids, glycolic acid, malic acid, tartaric acid, latic acid, citric acid, mandelic acid and salicylic acid. The optimized CE conditions were phosphate solution containing CTAB, CD and organic solvent as running buffer, sample injection 20 second by 0.5 psi and -15 kV as separation voltage at 25&#8451 ; with UV wavelength 200 nm. The seven HAs were separated well in less than 10 min. Compared with CE, LC-MS can offer a significant increase of sensitivity and selectivity. After optimization and validation, the simple and rapid analysis CE and LC-MS method was established and successfully applied to several commercial cosmetic products and biosamples.

PWe-049
11:10 – 12:20

Fast determination of phosphopeptides by nanoLC-MS/MS in biological samples

Rong-Chun Chen, Chi-Yu Lu

Department of Biochemistry, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Keywords:
derivatization ; phosphopeptide ; biological samples

Novel aspects:
Combination of fast digestion and derivatization to detect phosphopeptides in biological samples

Abstract:
Protein phosphorylation is one of the post-translational modifications that plays an important role in signal transduction. This modification of protein is also related to many chronic diseases, including hypertension, cancer and Alzheimer's disease. Mass spectrometry combined database searching is very effective for protein identification and modification site recognition. This study developed a convenient way for protein digestion by using microwave to shorten the digestion time. After digestion, chemical tag was introduced to label phosphorylated peptides via beta-elimination and Michael addition. This derivatization protocol could help phosphopeptides to increase the ionization efficiency and avoid the neutral loss of phosphate group in fragmentation process. Application of this strategy to fast detect phosphopeptides in biological samples (such as milk, plasma or urine) demonstrated workable.

PWe-050
13:30 – 14:40

Derivatization of lipoic acid for analysis by capillary liquid chromatography and mass spectrometry

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Keywords:
lipoic acid, derivatization, capillary liquid chromatography, matrix-assisted laser desorption ionization time-of-flight mass spectrometry

Novel aspects:
Microscale derivatization method to enhance the sensitivity of lipoic acid in UV absorption and ionization ability in mass spectrometry.

Abstract:
Lipoic acid (LA) is an essential cofactor for mitochondrial enzymes and ideal antioxidant present in prokaryotic and eukaryotic cells. It is capable of scavenging reactive oxygen species and regenerating other antioxidants such as vitamine C and glutathione. Direct analysis of LA presents obstacle because its lack of chromophores. This study employed a pre-column derivatizing method to enhance the sensitivity of LA in UV absorption and ionization ability in matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) . Dispersive liquid-liquid microextraction (DLLME) method is used as sample pretreatment method. All important parameters affected the extraction and derivatization of LA were investigated and optimized. The capillary liquid chromatography (CapLC) separation was carried out by using capillary C18 column with the mobile phase consisting of acetonitrile-0.1% formic acid. The linear response was ranged from 0.1 to 40 μM and 0.1 to 20 μM in standard solution and urine respectively, with a correlation coefficient of 0.999. The detection limits was 0.01 μM and 0.03 μM by CapLC-UV, and the detection limits was 0.008 μM and 0.02 μM by MALDI-TOF MS in standard solution and urine respectively. We successfully applied these methods to detect LA in dietary supplements and urine.

PWe-051
11:10 – 12:20

Highly-Selective Reaction Monitoring (H-SRM) with Automated Mass and Resolution Calibration in Testing of Anabolic Steroids in Equine Plasma and Urine

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Keywords:
Automated calibration, H-SRM, Anabolic Steroids

Novel aspects:
Automated calibration using lock-peaks to facilitate the use of highly-selective reaction monitoring (H-SRM) acquisition in the analysis of anabolic steroids in equine plasma and urine

Abstract:
Use of anabolic steroids in the horse racing industry is under increasing scrutiny and regulation. Numerous regulatory bodies have specified tolerance limits depending on the sex of the horse. Due to the fact that some steroids are naturally occurring and certain levels are allowed in stallions (males) as opposed to mares (females) , it is critical to precisely measure and monitor the levels of steroids in stallions and mares during competition. The traditional method of steroid testing involves detection, quantification, and confirmation using a triple quadrupole mass spectrometer. The matrixes monitored include plasma and urine, both of which can provide significant instrument and data interpretation challenges due to endogenous matrix interferences. Sample preparation and optimized chromatography can reduce matrix interferences. However, there are occasions when these options are not sufficient. To reduce matrix interferences, the use of highly-selective reaction monitoring (H-SRM) acquisition is encouraged. H-SRM acquisition uses a reduced peak width, such as 0.2 FWHM in Q1, and standard peak width of 0.7 FWHM in Q2. Reducing the peak width in Q1 reduces the potential of isobaric interferences. For this reason, we propose the use H-SRM acquisition, utilizing a peak width in Q1 of 0.2 to 0.1 FWHM as needed to reduce any matrix interferences in the testing of anabolic steroids in horse urine and plasma. The typical triple quadrupole mass spectrometer will lose significant signal intensity when the peak width is below 0.7 FWHM rendering H-SRM ineffective in reducing matrix interferences. The triple quadrupole instrument employed in this study utilizes hyperbolic quadrupole rods which enable the use of resolutions of 0.2 or even 0.1 FWHM with minimal decrease in signal intensity.

One potential issue with narrow peak widths in H-SRM is loss of sensitivity or selectivity due to minor mass shifts or resolution changes. These long term mass or resolution changes are often due to environmental conditions in the laboratory such as temperature fluctuations. Under unit mass resolution, peak width of 0.7 FWHM, small changes in mass or resolution are insignificant. However, as mentioned, with H-SRM small mass shifts or resolution changes can cause loss of signal or increase in background noise which affect ; sensitivity, limits of detection, reproducibility, and ultimately can generate misleading results. To compensate for changes in mass or resolution we will periodically perform an automated mass and resolution calibration check, and correction, during the analysis sequence. This procedure, using one or multiple lock-peaks, will automatically correct any mass drift or resolution changes that might occur over time.

Over the course of multiple weeks H-SRM calibration curves for testosterone, boldenone, and stanozolol in equine urine and plasma will be repeatedly analyzed using the method developed at the Pennsylvania Equine Toxicology and Research Laboratory. Sample analysis using automated re-calibration will be compared to the sample analysis run with a standard calibration performed at the start of the study. Sequential series of samples using standard calibration and automated re-calibration will be analyzed on the same instrument to eliminate possible instrument variability. The linearity, reproducibility, limit of detection, and accuracy of quantification for the three anabolic steroids will be used to assess the effectiveness of the automated re-calibration analysis versus that of standard calibration analysis. We propose that the use of H-SRM analysis with automated re-calibration is a highly selective, rugged, and reliable method of analyzing anabolic steroids in equine urine and plasma.

PWe-052
13:30 – 14:40

Selective enrichment of histidine-tagged proteins and peptides on MALDI plate

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Keywords:
histidine-tag, affinity, MALDI MS, Nanoparticles

Novel aspects:
A new type of magnetic nanoparticle-based affinity-mass spectrometric platform for rapid characterization of histidine-tagged proteins/peptides from complex samples was explored in this study.

Abstract:
Recombinant proteins expressed in bacterial cells are commonly tagged by a polyhistidine for the ease of purification. Conventionally, recombinant proteins tagged with a polyhistidine, i.e. his-tagged proteins, can be readily purified by metal ions (Ni (II) or Co (II)) immobilized affinity columns (IMAC) from complex cell lysates. In this work, we explored that aluminum oxide coated iron oxide magnetic nanoparticles (Al_2O_3@Fe_3O_4 MNPs) have high affinity toward polyhistidine, leading Al_2O_3@Fe_3O_4 MNPs to be suitable trapping probes for his-tagged proteins/peptides. We demonstrated the feasibility of using Al_2O_3@Fe_3O_4 MNPs as affinity probes for his-tagged proteins and peptide. Furthermore, taking advantages of the magnetic property of the affinity probes, the enrichment and isolation of target species can be realized on a matrix-assisted laser desorption/ionization (MALDI) plate with the assistance of a magnet. The species enriched by the affinity probes on the plate can be characterized by MALDI MS. His-tagged proteins, protein digests, and cell lysates were used as the model samples. High enrichment capability for his-tagged species can be achieved as high as two orders of magnitude. This approach provides the advantages including simplicity, high selectivity, high sensitivity (~ nM) , and short extraction time (~ 30 s) . Thus, we believe that this approach should be very suitable for rapid investigation of the expression of his-tagged recombinant proteins in prokaryotic organisms.

PWe-053
11:10 – 12:20

Determination of Fluconazole and 5-Fluoro Cytosine in human plasma and cerebrospinal fluid using protein precipitation and LC-MS/MS

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Keywords:
fluconazole, 5-fluoro cytosine

Novel aspects:
Bioanalytical method validation of fluconazole and 5-fluoro cytosine (5-FC) in human plasma and cerebrospinal fluid

Abstract:
A sensitive and robust method for the detection of fluconazole and 5-fluoro cytosine (5-FC) in human plasma and cerebrospinal fluid (CSF) was developed and validated. Stable isotopic internal standards of fluconazole and 5-FC were used as LC-MS internal standards. The method was validated over the calibration range of 0.195 µg/ml to 50.0 µg/ml using protein precipitation. Chromatography was developed on an Atlantis T 3 reverse phase column with a mobile phase of 0.1% formic acid and acetonitrile. The method was fully validated according to Food and Drug Administration (FDA) recommendations (good selectivity, carry-over less than 20 %, inter-and intra-assay precision and accuracy lower than 15 %) . Stock solutions of fluconazole and its internal standard were stable for up to 6 hours at room temperature. However stock solutions of 5-FC as well as its internal standard were unstable under the same conditions. The stability of both analytes in plasma and CSF for 4 hours was within FDA recommendations. Recovery, matrix effects and haemolysis were acceptable following FDA recommendations. On-instrument stability showed that the analytes are stable in extracted matrix for up to 72 hours in plasma and 48 hours in CSF. This method is usable for the routine detection of fluconazole and 5-FC in human plasma and CSF and may be useful in future clinical studies.

PWe-054

13:30 – 14:40

Studies on the selective binding of divalent metals to cyclodextrins by ESI mass spectrometry

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Keywords:

divalent metals, cyclodextrins, selective binding,

Novel aspects:

The selective binding of divalent metals to cyclodextrins is firstly studied using electrospray ionization mass spectrometry. The alkaline-earth di-ions showed different binding strength from transition metal di-ions.

Abstract:

Cyclodextrins (CDs) , composed of α-1,4-coupled D-glucose units, have the ability to form lots of inclusion complexes with a wide range of molecules in aqueous solution, which allow them to be applied in many areas such as enzyme mimics, catalysis and the encapsulation of drugs[1-4]. However, the study on the complexes of cyclodextrin with inorganic divalent metals by mass spectrometry is rarely reported.[5-7] The electrospray ionization mass spectrometry (ESI-MS) has been recognized as a useful tool for investigating the general area of molecular recognition and providing a powerful method for the analysis of various host-guest complexes and other non-covalent complexes in solution.

The most commonly used CDs include α-CD, β-CD, and γ-CD, which consist of 6, 7, and 8 D-glucose units, respectively. In this report, the selective binding of divalent metals to cyclodextrins was investigated. In the experiment, the α-, β-, γ-, or hydroxypropyl-β-cyclodextrins, and $MgCl_2$,$CaCl_2$,$BaCl_2$, $FeCl_2$, $CuCl_2$ or $ZnCl_2$ solution were mixed in a molar ratio of 1 : 10, respectively, and then reacted at room temperature for 12 hours. The evaluation of the binding selectivity of Mass spectrometric experiments were performed using Thermo Finnigan LTQ ion trap mass spectrometer equipped with an electrospray ionization source. The capillary voltage was set to 4.0 kV and the capillary temperature was maintained at 350&#8451 ; . The sample was introduced via a syringe pump at a flow rate of 5 μL/min.

The ESI-MS experimental results showed that all of the α-, β-, γ-, and HP-β-CDs can form 1 : 1 complexes $[CD+M]^{2+}$ (M represents divalent metal) with $MgCl_2$,$CaCl_2$,$BaCl_2$, $FeCl_2$, $CuCl_2$, $ZnCl_2$ in 80%/20% methanol/water solution, but can't form 1 : 2 complexes $[CD+2M]^{4+}$, revealing that the ion products is significantly influenced by the Coulomb interaction between charges rather than the size of cyclodextrin's interior cavity.

The binding of α, β, and γ-CD to divalent metal ions was further confirmed by collision-induced dissociation (CID) experiment. The collision energy was 30 *eV*. The fragmentation pathways for all $[CD+M]^{2+}$ were similar, which was irrelevant to the D-glucose units' number. The fragment ions were mainly $[CD+M-n\ glucose]^{2+}$ (n =1, 2, 3) . It is demonstrated the loss of neutral molecule (D-glucose units of 162 *m/z*) took place in all fragmentation process, which provides more evidences that the complexes are of the inclusion type.

For comparison, a competition experiment was also performed. A stoichiometry of cyclodextrin were mixed with Mg^{2+},Ca^{2+},Ba^{2+}, Fe^{2+}, Zn^{2+} , and Cu^{2+} cations respectively. The mixed solutions then incubated at room temperature for 12 hours to reach the equilibrium. From ESI mass spectra, it can be seen that the peak intensity for the complexes of cyclodextrins with Mg^{2+},Ca^{2+},Ba^{2+} is 10 times higher than those of Fe^{2+}, Zn^{2+} and Cu^{2+}, which indicates that the binding strength for former complexes is stronger than the latter. The mechanism for this difference will be further investigated later in this laboratory.

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PWe-055

11:10 – 12:20

Dual Lectin-based Enrichment Method of N-linked Glycopeptides Using Asymmetrical Flow Field-Flow Fractionation and Applications to Human Blood Serum

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Keywords:
Flow Field-Flow Fractionation, Glycoproteomics, N-linked glycoprotein, nanoLC-ESI-MS-MS

Novel aspects:
Intact forms of targeted N-linked glycopeptides from human blood serum were able to be enriched selectively, with high reproducibility.

Abstract:
Glycosylation of proteins plays an important role in many biological processes related to cell-cell interaction, cell signaling, and cell proliferation. Despite the crucial fact, there are some drawbacks when characterizing glycoproteins in biological samples since the amount of glycoproteins occupies as significantly low as 2-5% of total amount of proteins. On this account, enrichment of glycoproteome is a critical step in glycoproteomic study. Among the enrichment methods of glycoproteome, a lectin-based approach is widely used for its ability to isolate targeted glycoproteins selectively when combined with various analytical techniques. In this study, the lectin-based enrichment method was developed for glycoproteomics by using asymmetrical flow field-flow fractionation (AF4), a size sorting technique that separates biological components.
Prior to enrichment of glycopeptides in human serum sample, depletion of albumin and IgG, which are abundant proteins in human serum, was accomplished to minimize interferences in reaction between lectins and glycopeptides. After the depletion, raw serum sample was digested in consecutive order using two enzymes (chymotrypsin and trypsin) in order to increase the efficiency of proteolytic cleavage. After a dual enzymatic treatment, proteolytic peptides of serum were blended with a mixture of lectins. Then, with AF4, molecular mass-separation of glycopeptides-bound lectins was carried out. Subsequently, lectin-glycopeptide complexes were collected and treated using endoglycosidase (PNGase F) in an attempt to characterize N-linked glycoproteins using nanoLC-ESI-MS-MS.
To validate the efficiency of selective enrichment of glycopeptides, proteolytic peptides of alpha-1-acid glycoprotein (AGP), a typical N-linked glycoprotein standard, were used for selective enrichment of glycopeptides containing glycan complex of high mannose type by using Concanavalin A (ConA). AGP is reported to have five N-linked glycosylated sites and additionally, is post-traslationally modified to glycans, in which 20% was consisted of mannose. Thus five peptides containing the glycosylation site as a deamidation of asparagine (by PNGase F) were detected among tryptic peptides of AGP without detecting other peptides. As a result, we identified five endoglycosidically digested peptides of ConA fraction using nanoLC-ESI-MS-MS experiments.
The enrichment method was applied to human blood sera from control and lung cancer patients, in order to excavate the biomarker related to lung cancer. By using the same procedures with two lectins (WGA and SNA), WGA- and SNA-specific bound glycopeptides from human serum were collected during AF4 separation and treated by PNGase F in sequence. Deglycosylated peptides from each lectin-glycopeptide fraction were identified by using nLC-ESI-MS-MS and database search. Consequently, 71 WGA and 39 SNA of N-linked glycopeptides were identified.

PWe-056
13:30 – 14:40

A nanoprobe-based strategy combined with HILIC enrichment for targeted glycoprotein profiling

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Keywords:
nanoprobe, glycopeptide, profiling, HILIC

Novel aspects:
This study demonstrates a sensitive strategy for the discovery of glycosylation pattern of low-abundant serum proteins.

Abstract:
Introduction :
Glycosylation is the most prevalent protein modification and 50 % of proteins are suggested to be glycosylated based on the genomic information. Increasing evidences have shown that the significant change in protein glycosylation pattern is an important feature during oncogenesis. Here we present a sequential enrichment method integrating functionalized nanoprobe with hydrophilic interaction chromatography (HILIC) for glycopeptides characterization using MALDI-TOF MS analysis. Based on the sensitivity of nanoprobe for serum marker purification and the selectivity of HILIC for glycopeptides enrichment, we aim to establish a simple protocol for the analysis of low-abundant serum glycoprotein bypassing sample fractionation, immuno-depletion of abundant serum proteins and minimum sample loss. To demonstrate the general availability of this approach, prostate-specific antigen (PSA) , the widely used glycoprotein marker for prostate cancer diagnosis, was selected as model system.

Preliminary results:
Taking advantage of efficient affinity extraction by surface-functionalized magnetic nanoparticles (MNPs) and accurate MALDI-TOF MS readout, we present success on this nanoprobe-based immunoassay for simultaneous enrichment and quantitation of PSA from human serum. The present results showed the detection limit as low as 5 ng/ml of PSA in human serum, which is capable of detecting PSA in serum from patient with prostate cancer or benign prostate hyperplasia (> 10 ng/ml) . In addition, our methodology demonstrated good quantitation linearity of PSA (R^2 &#8786 ; 0.99) with high precision (<18 %) and accuracy (<10%) with dynamic range of 5 -100ng PSA spiked in healthy female serum. Analysis of the glycosylation pattern was performed by extraction of glycopeptide from tryptic PSA peptides using a homemade HILIC spin column. After the specific enrichment by HILIC, the removal of the unglycopeptides enhanced the detection sensitivity of heterogeneous glycosylated peptides in MALDI-TOF MS analysis. This two-step workflow has shown the detection of multi-glycosylation of purified PSA from seminal fluid. The sensitivity and specificity of this platform shows promise as an alternative tool for the study of glycoprotein. The application of this approach to quantitatively compare the PSA glycosylation profiles in benign prostate hyperplasia and patients with prostate cancer is currently ongoing.

PWe-057
11:10 – 12:20

Alteration of glycan profile during early-stage of mesenchymal stem cells differentiation

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Keywords:
glycan profile, mesenchymal stem cells, liquid chromatography/mass spectrometry, multivariate analysis

Novel aspects:
Glycan profiles can be used as a molecular index for discrimination of MSCs from differentiated cells.

Abstract:
Human bone marrow mesenchymal stem cells (MSCs) are multipotent cells that are capable of differentiating into several cell lineages, including bone, cartilage, and neural-like cells. Cell therapy with MSCs has been recently attracted attention for the treatment of difficult-to-treat diseases such as neurological disorders. For full-scale practical use of cell therapeutic products from MSCs, development of a method for distinguishing MSCs from differentiated cells is needed. Most proteins produced in eukaryotic cells are glycosylated, and the glycan profiles are altered in association with several biological events, including cell differentiation and proliferation. Therefore, the glycan profile can serve as a potential molecular index for distinguishing between MSCs and differentiated cells. In this study, we analyzed glycan profile alterations during early-stage of MSC differentiations by using liquid chromatography/mass spectrometry (LC/MS) and multivariate analysis (MVA) to evaluate the usefulness of the glycan profile as a molecular index.

When MSCs were differentiated neurally, morphological alteration was observed after 2 days, although MSCs remained unaltered by osteogenic differentiation for 2 days. *N*-linked glycan profiles from MSCs, neural-lineage cells, and osteogenically differentiated cells showed that the major glycans in these cells were high-mannose-type, pauci-mannose-type, hybrid-type, and complex-type bi-, tri-, and tetra-antennary glycans. By MVA of the MS data, the 3 cells were plotted at different positions, indicating the glycan profile alterations. Furthermore, the characteristic glycans of the differentiated cells were identified using S-plot analysis, which allows confirmation of the highest contributing variables for the pre- and post-groups. Significant increase was observed in the level of a sialylated complex-type glycan in the neural-lineage cells. The levels of the high-mannose-type, pauci-mannose-type, and non-fucosylated complex-type glycans were decreased in the neural-lineage cells. In contrast, in the osteogenically differentiated cells, the levels of fucosylated and sialylated complex-type glycans were increased ; however, the levels of high-mannose-type and pauci-mannose-type glycans were decreased. These results suggest that glycan profiles can be used as a molecular index for discrimination of MSCs from differentiated cells.

PWe-058 Data-dependent acquisition system for N-linked glycopeptides using MALDI-DIT-TOF MS

13:30 – 14:40

Masaki Murase, Hidenori Takahashi, Yosihiro Yamada, Sadanori Sekiya, Shigeki Kajihara, Shinichi Iwamoto, Koichi Tanaka

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Keywords:
N-linked glycopeptide, MALDI, automation, de novo sequencing, database search

Novel aspects:
Development of automatic structural analysis system of glycopeptides integrating data-dependent acquisition and MSn analysis by MALDI-DIT-TOF MS.

Abstract:

Introduction

Glycosylation is one of the most common protein post-translational modifications, as more than 50% of proteins in humans are glycosylated. Glycosylation has important roles in various biological processes and aberrant glycosylation is associated with numerous diseases.

Analysis of glycosylation using tandem mass spectrometry at the glycopeptide level can reveal glycosylation sites, glycan components and peptide sequences. However, the procedure is laborious due to its complexity. To support data analysis of N-linked glycopeptides, we previously reported the integrated approach, which combines our original *de novo* sequencing software with conventional protein database search.

In this study, we developed a data dependent acquisition system called LDIA (Logical Data-Influenced Acquisition) with MALDI digital-ion-trap (DIT) MS (DIT is a 3D-quadrupole ion trap driven by a rectangular wave high voltage) . We demonstrate structural analysis of N-linked glycopeptides with this system.

Methods

Human transferrin glycopeptide GP 1 ($[M+H]^+$: *m/z* 3682) which contains a disialylated biantennary N-glycan, was prepared with reverse-phase HPLC from its tryptic digest. 2,5-dihydroxybenzoic acid (DHB) was used as a matrix.

Mass spectra were acquired using MALDI-DIT-TOF MS. The MALDI-DIT-TOF MS instrument control software provides functions for automatic MALDI MS data acquisition such as sweet spot search and laser power tuning.

The LDIA module was implemented as an automatic data acquisition plug-in for the control software. The main functions of the plug-in include (1) determination of precursor ions using neutral loss of sugars from MS spectra, (2) determination of MS^3 precursor ions and deduction of glycan components by *de novo* sequencing from MS^2 spectra, (3) identification of peptides and glycosylation sites from MS^3 spectra. X!Tandem (Global Proteome Machine Organization) was used to identify peptides and glycosylation sites.

Results

An MS^1 spectrum of transferrin-GP 1 was acquired automatically using MALDI-DIT-TOF MS and showed fragmentation of the glycopeptides, especially dominant loss of sialic acids. The LDIA plug-in assigned glycopeptide ion peaks using glycan sequencing by neutral loss of sugars, and several of the highest intensity peaks were selected as MS^2 precursor ions. Sialylated ions were automatically removed so that they were not selected as precursor ions, because CID of such ions results in poor fragmentation.

An MS^2 spectrum of $[M+3H-2Sia]^+$ was acquired automatically and showed characteristic cleavage for N-linked glycopeptides by CID as triplet peaks, which were formed by (1) one HexNAc molecule binding with a peptide (pep + 203Da) , (2) a peptide containing ring-cleaved HexNAc (pep + 83 Da) , (3) a peptide containing no glycans. The LDIA module determined the triplet peaks as the precursor ions for MS^3 to identify the peptide sequence and the glycosylation site. Correct glycan components were also deduced by *de novo* sequencing combined with a result from MS^1 analysis.

MS^3 spectra from the triplet peaks were acquired automatically, and the peptide sequence and glycosylation site were correctly identified by X!Tandem.

From these results it was shown that the automatic analysis system was useful for structural analysis of N-linked glycopeptides.

PWe-059 SRM analysis of chondroitin sulfate terminal regions

11:10 – 12:20

Harumi Osago[1], Tomoko Shibata[2], Suguru Kuwata[3], Michihaya Kono[3], Nobumasa Hara[1], Kazuo Yamada[1], Yuji Uchio[3], Mikako Tsuchiya[1]

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Keywords:
chondroitin sulfate, glycosaminoglycan, selected reaction monitoring (SRM) , free terminal, linkage terminal

Novel aspects:
Sulfated mono- or disaccharides and hexasaccharides derived from free and linkage terminals of CS in cartilage were analyzed with SRM mode of MS/MS analysis.

Abstract:
<Introduction>
The glycosaminoglycan (GAG) consists of repeating disaccharides and shows high heterogeneity generated through modifications such as sulfation or epimerization along the backbone. GAG family is classified on the disaccharide unit into several groups such as unsulfated hyaluronan (HA) and the sulfated chondroitin/dermatan sulfate (CS/DS) , heparan sulfate/heparin (HS/HP) and keratan sulfate (KS) . GAGs are constitutively located on the extracellular matrix of many tissues such as cartilage. GAG lyase such as chondroitinase ABC liberates unsaturated disaccharides and saturated mono- or disaccharides derived from internal and free terminal regions of GAG, respectively. Followed byfurther alkaline reduction, unsaturated hexasaccharides are released from link protein as linkage terminal region. Generally, the internal unsaturated disaccharides have been target for the analysis of GAGs and we reported the simultaneous measurement of the saccharides derived from HA, CS/DS, HS/HP and KS by tandem mass (MS/MS) analysis.
Recently, structural changes of the terminal regions of GAGs in cartilage, such as changes of sulfated pattern of free terminal and shortening of the length of CS, have been implied in diseases or aging conditions. In this study, we tried to establish the quantitative analysis conditions of terminal saccharides ofCS, such as GalNAc, GlcA/IdoA-GalNAc and ΔUA-GalNAc-GlcA-Gal-Gal-Xyl-ol, with selected reaction monitoring (SRM) mode of MS/MS analysis.
<Abbreviation>
GalNAc : N-acetyl-galactosamine, GlcA : glucuronic acid, IdoA : iduronic acid, ΔUA : 4,5-unsaturated uronic acid, Gal : galactose, Xyl : xylose
<Methods>
Commercially available CS chain, aggrecan and porcine articular cartilage solubilized with collagenase type II were digested with chondroitinase ABC. For linkage terminal analysis, digests were further alkaline reduced.
LC was done onHypercarb (Thermo Scientific) with the ammonium bicarbonate buffer (pH10-11) / acetonitrile system. MS analysis was done with ESI-QqQ (AB SCIEX, API3000) in the negative mode.
<Results and discussion>
By LC-MS analysis of the CS digest, the signals corresponding to the free terminal saccharides, mono- or disulfated GalNAc and monosulfated GlcA/IdoA-GalNAc were detected at *m/z* 300, 380 and 476, respectively. The MS/MS analysis of these ions detected *m/z* 97 ion corresponding to [HSO_4] and other product ions matching the fragments of these saccharides. The *m/z* 97 ion was selected for their SRM transitions. The signal at *m/z* 476 had 2 peaks. MS/MS analysis of each peak detected the product ions known to be specific to the unsaturated disaccharide containing 4- or 6-sulfated GalNAc of CS, indicating the presence of 4- or 6- sulfated GalNAc at the free terminal. Unsulfated form of GalNAc and GlcA/IdoA-GalNAc were not detected.
By MS analysis of alkaline-reduced aggrecan digest, the signals corresponding to the linkage terminal saccharides of CS, non- or mono- or disulfated ΔUA-GalNAc-GlcA-Gal-Gal-Xyl-ol were detected at *m/z* 506, 545.5 and 585.5, respectively. MS/MS analysis of these ions showed fragmentation patterns similar to those of the hexasaccharides reported previously. The product ion for SRM transition of unsulfated ΔUA-GalNAc-GlcA-Gal-Gal-Xyl-ol was selected at *m/z* 175 corresponding to glycoside bond cleavage ion C_1. Other two targets generated the product ion at *m/z* 97, which was selected for their SRM transitions.
By LC-MS/MS analysis of the porcine articular cartilage digest, using SRM mode with the specific product ions selected as described above, several saccharides derived from both terminals of CS (2 monosaccharides, 1 disaccharide and 3 hexasaccharides) were newly detected in addition to the previously detected 8 internal disaccharides.Quantifying CS saccharides of the both terminals together with internal region may be useful to detect alterations of the GAG in diseases or aging conditions.

PWe-060
13:30 – 14:40

Novel Bioinformatics Tool: Interpretation of Glycan Mass Spectra with Metal Adducts and Multiple Adduct Combinations

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Keywords:
Glycan, Structual Interpretation

Novel aspects:
Development of bioinformatics tool for support of glycans with metal adducts and multiple adduct combinations.

Abstract:

Introduction

Previously we had presented a bioinformatics tool for automated structural interpretation of glycan MS/MS and MS^n data. This was limited to characterizing glycans with single adduct of H and Na ($[M+H]^{+1}$, $[M-H]^{-1}$, $[M+2H]^{+2}$, ($[M+2Na]^{+2}$ etc.) . However, the ionization of glycans by mass spectrometry results in the formation of several different adducts. These adducts maybe present as single adduct or combination of multiple adducts. Here we expand our bioinformatics tool, SimGlycan, to support Li and K adducts as well as combination of multiple adducts such as Na + H, Li + H, Na+K etc. In order to demonstrate the utility of the software, a combination of permethylation and MSn are used to characterize glycans derived from bovine fetuin and human IgG.

Methods

Bovine fetuin and human IgG were reduced, alkylated and enzymatically digested. Samples were evaporated to dryness and re-suspended in 25 mM ammonium bicarbonate buffer (pH~8) . Upon re-suspension PNGase F was added to release the glycans. Released glycans were permethylated as described previously. All MS^n experiments were carried out on a Thermo Scientific Velos Pro linear ion trap mass spectrometer using direct infusion into a nanoelectrospray source. Data analysis was performed using SimGlycan software from PREMIER Biosoft.

Abstract

Studies have shown that glycans are very susceptible to the effects of salts and other compounds. In most cases small amounts of sodium and alkali metals are added to improve ionization efficiency. However, introduction of these adducts can result in spectra with precursors containing multiple adducts in different combinations which complicate interpretation of observed peaks to identify the structure. We have expanded our bioinformatics tool to support characterization of glycans with Li and K adducts and combinations of multiple adducts such as Na + H, Li + H, Na+K to name a few. In order to test the performance of the software to handle multiple adducts, glycans released from bovine fetuin were chosen. This was an ideal system to test the capability of software because the glycan content of fetuin has been characterized in depth.

Ion trap MS profile was acquired for permethylated glycans of bovine fetuin and specific precursors with different adducts and combination of adducts were targeted for MS/MS and MS^n. Data were imported into SimGlycan software for structural characterization and when needed structural isomer differentiation. SimGlycan characterized glycans were verified using manual assignment and previously published data. We were able to correctly identify and assign structures to the MS/MS and MS^n spectra using this software confirming what we had assigned previously via manual assignment.

We further extended this to glycans released from Human IgG. This is an area of interest as IgG are involved in human circulation as part of the humoral immune response and the changes in N-glycosylation of IgG associate with various diseases and affect the activity of therapeutic antibodies and intravenous immunoglobulins. Using a combination of permethylation and MS^n more than 30 structures were identified. All of the targeted glycans contain multiple adducts and SimGlyan was used in structural interpretation.

PWe-061
11:10 – 12:20

Determination of Extensive Glycosylation on Glycoproteins and Glycolipids in High-density Lipoprotein using Mass Spectrometry

Jincui Huang, Hyeyoung Lee, Nancy Rivera, Angela M Zivkovic, Jennifer Smilowitz, Bruce J German, Carlito B Lebrilla

University of California, Davis

Keywords:
Mass Spectrometry, HDL, Sialic Acid, Glycan-microheterogeity

Novel aspects:
Extensive site-specific glycosylation on HDL protein complexes and glycolipid analysis indicate that HDL is potentially a negatively charged particle.

Abstract:

Introduction

High-density lipoprotein (HDL) is the most abundant lipoprotein that enables lipids to be transported within the water-based bloodstream. Lipoproteins are macromolecular complexes with a surface envelope of amphipathic phospholipids, free cholesterol, apolipoproteins, and the associated proteins together with an inner core constituting mainly triglycerides (TG) . HDL has the highest protein content compared with other lipoprotein groups (VLDL, LDL, and IDL) , identified as a strong inverse predictor of rick of cardiovascular disease. The function of HDL in health and disease is largely relied on the interactions of surface proteins with cell membrane receptors, enzymes, and lipid-transport proteins. Glycosylation, one of the most common post-translational modifications, plays important roles in cell-cell recognition and communication. The glycosylation of HDL however has not been well explored despite the presence of glycoproteins and glycolipids. Using systematic glycomics and glycoproteomics approaches, we determined for the first time protein and site-specific glycosylation and lipid glycosylation in HDL.

Method

N-linked glycans were released from 60 µL HDL (triplicates) using PNGase F. The released N-glycans were cleaned up via solid phase extraction (SPE) procedure using graphitized carbon cartridges (GCC) . Pronase E was covalently coupled to CNBr activated sepharose beads via coupling chemistry, followed by adding 100 µL HDL and incubated at 37 °C for 18 h. The glycopeptide digest was desalted and enriched via SPE procedure using GCC. Extraction of gangliosides was performed by mixing water, methanol and chloroform with 100 µL HDL prior to centrifuging. Supernatant was collected, followed by clean-up procedure via SPE using C 8 cartridges. MS and MS/MS analyses of glycans, glycopeptides and gangliosides were achieved via nano-LC/Q-TOF MS with a nano-LC column integrated in a micro-chip packed with porous graphitized carbon for glycan/glycopeptides and reverse phase for gangliosides, respectively.

Preliminary data

HDL proteomic results revealed a number of apolipoproteins, Apo-A 1 as the most abundant one, and the associated plasma proteins approximately 60% of which are glycosylated.

Glycans were separated with a PGC stationary phase that is incorporated into an HPLC-microchip with high sensitivity and reproducibility. On average, our nano-LC method was able to resolve and identify over 60 N-linked glycan compound peaks with over 20 distinct N-linked glycan compositions from HDL. Each of the identified compositions included two or more peaks corresponding to structural and/or linkage isomers. Sialylated glycans as Complex type with bi- or tri- antenna were found to be the predominant glycan type. Interestingly, a bi-antennary glycan with two sialic acids was the most abundant glycan species. The structures of N-glycans from milk glycoproteins were further elucidated by tandem MS.

Comprehensive glycan-microheterogeneity of HDL glycoproteins was achieved by nano-LC/Q-TOF MS and in-house Glycopeptide Finder as a bioinformatics tool. Nonspecific protease digestion enabled the representation of all glycans attached to each N- or O-glycosites with a short peptide tag. In all, over 50 glycopeptides (identified based on composition and including isomers) corresponding to 20 glycosites were observed and determined in HDL protein mixture. The glycopeptides were a mixture of N-linked glycopeptides (mainly complex and sialylated glycans) and O-linked glycopeptides (sialylated) . Results were comprehensive as detailed glycan microheterogeneity information was obtained.

Ganglioside profiles showed that GM 3 (monosialoganglioside, NeuAc23Gal14GlcCer) and GD 3 (disialoganglioside, NeuAc28NeuAc23Gal14GlcCer) are abundant ions in human HDL. A 75 % GM 3 and 25 % GD 3 distribution was observed. Both GM 3 and GD 3 are composed of heterogeneous ceramide lipids, including d34 : 1, d39 : 1, d40 : 1 and d41 : 1.

PWe-062 Quantitative analyses of phosphatidylcholine hydroperoxides in oxidized lipoproteins by LC/MS

13:30 – 14:40

Shu-Ping Hui[1], Toshihiro Sakurai[1,2], Seiji Takeda[1], Shigeki Jin[1], Hirotoshi Fuda[1], Takao Kurosawa[3], Hitoshi Chiba[1]

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Keywords:
Lipid hydroperoxide, Oxidized lipoproteins, LC/MS

Novel aspects:
Unstable phosphatidylcholine monohydroperoxides in human lipoproteins were measured by liquid chromatography/mass spectrometry using nonendogenous 1-palmitoyl-2-heptadecenoylphosphatidylcholine monohydroperoxide as an internal standard.

Abstract:

Background

Phosphatidylcholine hydroperoxide (PCOOH), a primary oxidation product of PC, has been the focus of lipid peroxidation. In recent years, liquid chromatography (LC)/mass spectrometry (MS) has become a powerful tool for structurally identifying PCOOH. We previously reported a quantitative LC/MS method for PC-OOH using a synthetic internal standard of hydroperoxide [1]. Unfortunately, we occasionally observed interference by unspecified substance (s) in plasma samples with this internal standard. To solve this problem, we established an improved LC/MS method with a new synthetic internal standard and applied it in quantitative analyses of PCOOH in human low-density lipoprotein (LDL) and high-density lipoprotein (HDL) during copper-mediated oxidation [2].

Methods

1-Palmitoyl-2-linoleoylphosphatidylcholine monohydroperoxide (PC 16:0/18:2-OOH), 1-stearoyl-2-linoleoylphosphatidylcholine monohydroperoxide (PC 18:0/18:2-OOH), and 1-palmitoyl-2-heptadecenoylphosphatidylcholine monohydroperoxide (PC 16:0/17:1-OOH as IS) were chemically synthesized. We prepared oxidized LDL (oxLDL) and oxidized (oxHDL) by incubating native LDL (nLDL) and native HDL (nHDL) from human plasma (n=10) with $CuSO_4$ for up to 4 h. For quantitative analysis, 2 mL nLDL, nHDL, oxLDL, or oxHDL was extracted with chloroform. The residue was dissolved in 300 μL methanol and a 10-μL aliquot was injected into our quantitative LC/MS system. Reversed-phase LC separation was conducted on a reversed-phase column (Hypersil Gold C8, 50 mm×2.1-mm inner diameter, 5 μm; Thermo Fisher Scientific, Waltham, MA, USA) at 60 °C. The mobile phase consisted of three solvents: solvent A was 10.0 mM ammonium acetate aqueous solution, solvent B was acetonitrile, and solvent C was 2-propanol. The gradient was programmed as follows: 0.00-2.00 min 100% solvent A; 2.0-5.00 min 10% solvent A and 90% solvent C; 5.0-8.00 min 100% solvent C; 8.0-10.00 min 20% solvent A and 80% solvent B. The flow rate was 0.3 mL/min. Quantitative analyses were performed using a Prominence LC system (LC-20AD unit and SIL-20A autosampler; Shimadzu, Kyoto, Japan) and a TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization probe (Thermo Fisher Scientific, Waltham, MA, USA). The scan time was 0.333 s in positive ion mode. The electrospray ionization inlet conditions were as follows: ion source voltage ±3.0 kV; vaporizer temperature 350 °C; sheath gas (nitrogen) pressure 50 psi; auxiliary gas pressure 15 psi; capillary temperature 300 °C; Argon was used as the collision gas at 1.5 mTorr.

Results

The calibration curves for synthetic PC 16:0/18:2-OOH and PC 18:0/18:2-OOH, which were obtained by direct injection of the IS into the LC/MS system, were linear throughout the calibration range (0.8-12.8 pmol). Within-day and between-day coefficients of variation were less than 10%, and the recoveries were between 86% and 105%. The limit of detection (LOD) and the limit of quantification (LOQ) were determined using synthetic standards. The LOD (signal-to-noise ratio 3:1) was 0.01 pmol, and the LOQ (signal-to-noise ratio 6:1) was 0.08 pmol for both PC 16:0/18:2-OOH and PC 18:0/18:2-OOH. With use of this method, the concentrations of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH in the lipoprotein fractions during copper mediated oxidation were determined. The time course of the PC 16:0/18:2-OOH and PC 18:0/18:2-OOH levels during oxidation consisted of three phases. For oxLDL, both compounds exhibited a slow lag phase and a subsequent rapidly increasing propagation phase, followed by a gradually decreasing degradation phase. In contrast, for oxHDL, both compounds initially exhibited a prompt propagation phase with a subsequent plateau phase, followed by a rapid degradation phase. The analytical LC/MS method for phosphatidylcholine hydroperoxides might be useful for the analysis of biological samples.

References

1. Hui SP, et al. J Chromatogr B (2010) 878 : 16771682
2. Hui SP, et al. Anal Bioanal Chem (2012) DOI 10.1007/s00216-012-5833-x

PWe-064
13:30 – 14:40

In situ analysis of soybean and nuts by Probe Electrospray Ionization mass spectrometry. Sequential ionization of carbohydrates and lipids

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[1]University of Buenos Aires, [2]Yamanashi University, [3]Ehime University

Keywords:
Lipids, carbohydrates, soybean, nuts, tissue

Novel aspects:
PESI MS applied to direct analysis of lipids and carbohydrates in intact soybean and nuts. Potential application of PESI MS to nuts tissue imaging.

Abstract:
The probe electrospray ionization (PESI) is an ESI-based ionization technique that generates electrospray from the tip of a solid metal needle.[1] In the present work, we describe the PESI mass spectra obtained for *in situ* measurement of soybeans and several nuts (peanuts, walnuts, cashew nuts, macadamia nuts, pistachios and almonds) using different solid needles as sampling probes. For comparative study ESI MS and PESI MS analyses were performed for methanolic extracts from the seeds. Because seeds are rich in phospholipids, strong signals of phosphatidylcholine, phosphatidylethanolamine and triacylglycerols of different fatty acid residues were detected as major ions in the positive ion mode. Lipids were observed mostly as adducts with Na^+ or K^+. No significant differences were observed between ESI-MS and PESI-MS. The great advantage of PESI-MS is that no sample preparation is needed and minimum volume of organic solvents is required.
In order to find the optimal experimental conditions for seeds analysis by PESI MS some variables were evaluated. (i) The metal materials and needle diameters were evaluated. (ii) A typical protocol for lipids extraction from seed tissues was used and the mass spectra obtained for extracts were compared with those obtained *in situ* PESI analysis. (iii) For direct PESI analysis, several solvents were used to make the sample surfaces wet for the extraction of the components from the samples. (iv) Two methods were used to acquire the PESI mass spectra : the single-shot mode and the continuous-shot mode. In the former, the droplet was left to be electrosprayed until the electrospray current decreased to zero and in the latter the droplet was periodically renewed.
It was found that PESI MS is a valuable approach for in situ lipid analysis of seeds. Additionally, PESI mass spectrum is not probe material dependent. However, by modifying the titanium wire surface with pentafluorophenyl-triethoxysilane to be hydrophobic, time-dependent mass spectra could be obtained when single-shot mode is adopted allowing sequential analyses of carbohydrates and lipids.

[1] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki, Rapid Commun. Mass Spectrom. 21, 3139 (2007) .

PWe-065
11:10 – 12:20

Comprehensive Lipid Profiling with Multiplexed Precursor Ion Scanning using the New AB SCIEX QTRAP® 4500 LC/MS/MS System

Tomoko Nembai, Toshiyuki Yamazaki, Noriko Fueki, Kaoru Karasawa

K.K.ABSCIEX

Keywords:
New instrument, Lipid, Profiling, High-throughput

Novel aspects:
As QTRAP® 4500 has fast scan speed and positive/negative switching speed ; it took only a few minutes to complete multiple experiments.This advantage also resulted in saving consumption of samples.

Abstract:

[Purpose and Methods]

Cellular lipids play an important role in biological functions which include the formation of cell membranes, regulation, energy metabolism, and signal transactions.

Lipids have highly structural diversity due to the complex combination of hydrophobic acyl chain molecular species ; hence it is significant to characterize and quantify diverse types of lipids, and also to analyze variability when understanding various aspects of physiological phenomena.

Mass spectrometry has emerged as a powerful analytical tool for lipidomics research. To detect and identify lipids selectively and comprehensively, multiple specific precursor ion and neutral loss scan mode experiments which based on lipid classes and fatty acid chain fragments are widely used. However, it is sometimes difficult to do comprehensive profiling to detect trace amount of component due to scan speed or sensitivity limitation. Moreover, identification of numerous types of lipids is very complicated.

The new AB SCIEX QTRAP® 4500 System, which offers the very fast quadrupole scanning, up to 12000 Da/s, achieving a wide mass range survey scans from 5 to 2000 Da in milliseconds and delivering improvements in sensitivity, makes it possible to overcome the limitations and difficulties in conventional systems. Improved performances enable multiple precursor ion and neutral loss scan mode experiments in both positive and negative polarities with high scan speed and high sensitivity simultaneously. What is more, post-acquisition data processing can be fully automated using LipidView™ Software which allows lipid investigator the ability to easily find, identify and interpret lipid species from complicated mass spectrometry data.

In this work, asolectin from soybean (Sigma-Aldrich Inc.) and other lipid mixtures were used as test samples.

A total of 80 combinations of Precursor ion scan (PIS) and Neutral loss scan (NL) experiments were acquired in a single method with positive and negative polarity switching.

Samples were diluted with 0.1 % formic acid/ 5 mM ammonium formate in isopropanol and introduced by direct infusion. These combinations were based on lipid class-specific fragment ions or fatty acid-specific ones up to 22 carbon chain lengths.

For post-acquisition data processing, LipidView™ Software was used for identification and quantification of lipids.

[Results]

As QTRAP® 4500 has 1000 Da/sec scan speed and 50msec positive/negative switching speed, it took only 10 minutes to complete 80 experiments (accumulation cycle 20 times) , whilst conventional LIT system needs 60 minutes.

This speed advantage also resulted in saving consumption of samples. To acquire equal or better quality of data, QTRAP® 4500 needed only 100 uL of sample needs and it was 1 / 5 amount of current system.

The acquired data was processed by the LipidView™ Software.

In asolection, phosphatidyl ethanolamine (PE) 34 : 2 and phosphatidyl inositol (PI) 34 : 2 constructed in the fatty acid 16 : 0 and 18 : 2 were mainly identified.

[Conclusions]

The AB SCIEX QTRAP® 4500 System provides versatility, sensitivity, specificity and speed required for high-throughput lipid profiling of complex biological extracts by direct infusion.

These improvements enable us analysis of scarce sample, because it requires less amount of sample than conventional system.

When combined with LipidView™ Software, this complete hardware and software package offers unique data acquisition strategies.

PWe-066 Comprehensive fatty acid profiling using a single lipidomic approach

13:30 – 14:40

futoshi sato[1], Giorgis Isaac[2], Amrita Cheema[3], Kieran J Neeson[2], Jeff Goshawk[2], Jayne Kirk[2], Andrew Baker[2], Alan Millar[2], Albert J Fornace Jr [3], Jim Langridge[2], Giuseppe Astarita[2]

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Keywords:
Lipids, UPLC, Ion mobility

Novel aspects:
Comprehensive screening and fingerprinting of fatty acid composition for phenotypic identification and comparative lipidomic analysis.

Abstract:

Introduction

Fatty acids are present in biological samples both in unesterified (free) and esterified forms. Their analysis usually requires multi-step procedures, which includes enzymatic or chemical hydrolysis and their subsequent derivatization. These laborious procedures not only affect the sensitivity of detection, but also lead to a loss of information regarding the actual fatty acyl composition of different complex lipid classes (e.g., glycerolipids, glycerophospholipids, sterol lipids and sphingolipids) . Here we present a robust method for the simultaneous profiling of free and esterified fatty acids from various biological samples using ultra performance liquid chromatography (LC) coupled with ion mobility mass spectrometry.

Methods

We used - 2 µm particle size LC with an ion mobility TOF to discriminate lipids based on the different cross collision sections. Within the same chromatographic run, the mass spectrometer was set to apply alternate collision energies to the transfer collision cell between low and elevated collision energy ramp, with the latter inducing lipid fragmentation. As the fragmentation occurs post ion mobility separation, the precursor at low energy share drift time with its fragments from the elevated energy scan. Post-acquisition, we extracted and visualized the fatty acid information contained in the spectra using novel informatics solutions for neutral loss or precursor ions survey.

Abstract

We analyzed total lipid extracts from various animal tissues using sub- 2 µm particle size LC coupled with ion mobility. The post ionization separation by ion mobility allowed to increase the peak capacity and the specificity of identification. Lipid ions with different degree of unsaturation and acyl length migrated with characteristic mobility times due to their unique interactions with the nitrogen gas in the ion mobility cell. By alternating low and elevated collision energy in a data independent acquisition mode, we were able to simultaneously collect both complex lipids and fatty acyl information. Fatty acyl groups were detected as either neutral loss in positive mode or as charged ions in negative mode. The post-acquisition analysis allowed to differentiate the fatty acyl content and distribution in various lipid classes according to the different biological samples analyzed. Fatty acyl composition could be visualized as bidimensional maps (drift time versus retention time) , which translated in molecular fingerprints of the various tissues

PWe-067
11:10 – 12:20

MALDI-TOF MS analysis of phosphatidic acid using phosphate capture molecule, Phos-tag

Mai Urikura[1], Yoshino Kondo[1], Jun-ichi Morishige[1], Tamotsu Tanaka[2], Kiyoshi Satouchi[1]

[1]Fukuyama University, Fukuyama, Japan, [2]University of Tokushima Graduate School, Tokushima, Japan

Keywords:
MALDI-TOF MS, Phos-tag, phosphatidic acid

Novel aspects:
We developed the method for PA determination using phosphate capture molecule, Phos-tag.

Abstract:

[Background]

Liquid chromatography mass spectrometry coupled with electrospray ionization (ESI LC-MS) has been established as a method for identification and quantification of compounds with low molecular mass, and now it has been prevalent for lipidomics in the biological system. Another soft-ionizing method, matrix-assisted laser desorption and ionization mass spectrometry coupled with a time-of-flight mass spectrometry (MALDI-TOF MS) has been primarily established for the analysis of compounds with high molecular mass such as protein. Recently, MALDI-TOF MS have been evaluated as a useful methodology in lipid research due to its simplicity and high performance. Compared with ESI LC-MS, advantage of MALDI-TOF MS is free from carry-over contamination and deterioration of sensitivity due to dirt of ion source. On the other hand, however, it requires purification step if objective molecule is a minor component in the samples.

In the present session, we would like to show the MALDI-TOF MS analysis of phosphatidic acid (PA) , a potential anti-ulcer phospholipid produced by phospholipase D (PLD) in cruciferous vegetables such as cabbage.

[Experimentals]

Cabbage juice was obtained by squeezing row cabbage leaves and Phos-tag was obtained from MANAC Inc. (Fukuyama) . Cabbage juice was incubated with egg yolk or soybean lecithin. The products were extracted by Bligh and Dyer procedure and analyzed by MALDI-TOF MS using Phos-tag coordinated with the single isotopic zinc, ^{68}Zn. Phos-tag was coupled chemically to Toyopearl resin and the bead was used for isolation of PA.

[Results and Discussion]

PA is a minor membrane phospholipid constituting less than one hundredth of total phospholipids. PA is an intermediate of phospholipid synthesis, but it has been recently recognizing that PA is biologically active phospholipid, such as second messenger upon cell stimulation and an anti-ulcer protector in digestive tracts. Accordingly, demands to quantify a small and transient amount of PA have been increasing. PA is monoester type of phospholipid and has two negative charges in phosphate, thus, three positive charges are necessary to produce a single positively complex ion. As a result, multiple ions due to additions of protons, sodium and potassium ions, and their combinations are detected in a mass spectrum of PA in positive mode. This is the problem for analysis of molecular species, where PA comprised several molecular species with many fatty acids. Zinc coordinated complex, Phos-tag, was synthesized by referring the spatial configuration of zinc metals in the active site of alkaline phospahatase. This reagent can capture phosphate compound specifically and dose-dependently. Phos-tag can unify adducts of PA to $[PA^{2-}/Phos\text{-}tag^{3+}]^{+}$ in positive mode of MS. It contributes not only to the simplification of mass spectra, but also the increase in detection efficiency.

Using ^{68}Zn Phos-tag, PA formation by the action of PLD from cabbage juice can follow in a short time by MALDI-TOF MS without purification step. Molecular species of PA produced from egg yolk lecithin were 1-saturated-2-unsaturated species like palmitoyl/oleoyl (16:0/18:1) PA, whereas those from soybean lecithin were 1,2-diunsaturated species like linoleoyl/linoleoyl (18:2/18:2) PA in addition to 1-saturated-2-unsaturated species.

For a trace amount of PA, suppression effects of co-existing other phospholipids are unavoidable. To overcome, Phos-tag Toyopearl bead is available. An equal amount of mixture of phospholipids, phosphatidylcholine (PC) and PA was incubated with the bead in methanol. PC, diester type of phospholipids, is eluted from the bead thoroughly, whereas PA having monoester type of phosphate interacts with Phos-tag. The retained PA in the bead was recovered by addition of 0.01 M HCl in methanol. Using Phos-tag Toyopearl for isolation, technique for quantitation of PA by MALDI-TOF MS has been under investigations.

PWe-068 Novel Non-targeted Lipidomic Quantitation using HILIC-HPLC/MS

13:30 – 14:40

Eva Cifkova, Miroslav Lisa, Michal Holcapek

University of Pardubice, Pardubice, Czech Republic

Keywords:

Lipidomics ; lipid class ; quantitation ; HILIC-HPLC/MS

Novel aspects:

Non-targeted lipidomic quantitation of lipid classes using hydrophilic interaction liquid chromatography and mass spectrometry.

Abstract:

The identification and quantitation of all lipids in complex biological tissues is the first step towards the understanding how lipids function in a biological system and the elucidation of the mechanism of lipid-related diseases including obesity, atherosclerosis, cancer, cardiovascular diseases, etc. HPLC/MS has the highest potential to obtain comprehensive information about the whole lipidome, but the reliable quantitation of all lipid classes is a difficult and challenging task. Our optimized HILIC-HPLC/MS enables the fractionation of 19 lipid classes in a wide range of polarities [J. Chromatogr., A 1218 (2011) 5146] . The goal of current research work is the development of new non-targeted method for the quantitation of main lipid classes and also lipid species within these classes using a single platform. For this purpose, the novel non-targeted lipidomic quantitation was developed and results were compared with the conventional approach with multiple selected reaction monitoring (SRM) scans on triple quadrupole analyzer. Concentrations of individual lipid classes were obtained by the peak integration in the HILIC mode multiplied by response factors calculated for individual classes and correlated with sphingosyl ethanolamine (d17 : 1/12 : 0) as an internal standard. In comparison to conventional SRM approach, our method allows to quantify all separated lipid classes without the definition of individual SRM transitions. Subsequently, fractions of individual lipid classes are collected and used for off-line 2D HILIC x RP-HPLC/MS analysis. RP-HPLC separation in the second dimension provides more detailed qualitative and quantitative information about lipid species within these classes including the isobaric differentiation. Our non-targeted quantitation is an appropriate tool for the comprehensive lipidomic characterization of multiple lipid classes and the determination of lipid composition differences between healthy and disease tissues.

This work was supported by the grant project No. 206/11/0022 (Czech Science Foundation).

PWe-069
11:10 – 12:20

Shotgun analysis of lipid extract from mouse brain by using comprehensive LC/MS

Jisun Yoo, Eun Jung Bae, Taeseong Park, Young Hwan Kim

Korea Basic Science Institute, Ochang, Korea

Keywords:
lipidomics, LC/MS,

Novel aspects:
A method of combined profiling and MS/MS using UPLC coupled with mass spectrometry was developed for the comprehensive analysis of lipid extract from mouse brain.

Abstract:
Lipidomics is a rapidly expanding research field in which multiple techniques are utilized to quantify the precise chemical constituents in a cell's lipidome and identify their cellular organization. The results obtained by lipidomics provide the information on their biochemical mechanisms through which lipids interact with each other and with crucial membrane-associated proteins. LC/MS has played an important role in the detection and identification of lipids. Especially, the ultra-performance liquid chromatography (UPLC) is now widely used and applied to not only lipid analysis and which have a high efficiency, resolving power, and sensitivity, and rapid analytic ability. In this study, a method for lipid profiling using UPLC coupled with mass spectrometry has been demonstrated for the analysis of mouse brain. For the analysis of lipids in biological samples, the use of MS^E data results in multiple levels of information which is extremely useful when trying to confirm the identity of a specific compound. For the determination of the repeatability, linearity and recovery, the ratio of the peak areas of the endogenous lipid and the corresponding internal standard was calculated. On the other hand, MS^E acquires all relevant information in both low energy and high energy acquisitions during the same analysis, and in the subsequent data processing available information on fragmentation pathways, diagnostic precursor and neutral losses allowed us to determine the polar head group and fatty acid composition in a relatively fast and efficient manner.

PWe-070
13:30 – 14:40

Top-Down Analysis of Lipoproteins by On-Line Field-Flow Fractionation and ESI-MS-MS

Ki Hun Kim, Ju Yong Lee, Myeong Hee Moon

Yonsei University, Seoul, Korea

Keywords:
lipoprotein, lipid analysis, field-flow fractionation

Novel aspects:
By using on-line cAF 4 -ESI-MS-MS, lipoprotein particles are separated by sizes and various lipids contained in HDL and LDL can be directly anayzed with structural identification by top-down approach.

Abstract:
Lipoproteins are globular particles composed of lipids and proteins in blood serum and their roles include transferring fats and cholesterols through blood vessels throughout the body. Especially, it has been known that smaller and denser low density lipoprotein (LDL) and low levels of high density lipoprotein (HDL) are risk factors of atherosclerosis and other cardiac diseases. Among lipoprotein components besides cholestrol, phospholipids (PLs) are a major lipid class composed of various molecular categories differed by the polar head groups and the lengths of acyl chains along with the degrees of unsaturation in acyl chain. For the analysis of lipoprotein components, it requires an isolation/fractionation of HDL and LDL particles from blood sample and these are commonly carried out by using density gradient ultracentrifugation (DGU) , gel-based electrophoresis, and size exclusion chromatography (SEC) . However these techniques have minor drawbacks such as the requirement of large amount of sample for DGU and the possible sample loss due to the interaction between lipoprotein particles with gel matrix for the last two methods.

Flow field-flow fractionation (FlFFF) is a technique capable of separating nanoparticles or proteins based on hydrodynamic size differences in an open channel by the simultaneous movement of a migration flow and a crossflow, where the crossflow is applied perpendicular to the migration flow. FlFFF has been utilized for the size determination of HDL and LDL from blood serum samples of CAD patients and the collected HDL and LDL particles can be further analyzed for the profiling of phospholipids by nanoflow liquid chromatography-electrospray ionization-tandem mass spectrometry (nLC-ESI-MS-MS) . This presentation introduces the top-down analysis of various lipid molecules contained in lipoprotein particles from plasma sample using a direct interface of the chip-type asymmetrical FlFFF (cAF 4) , a miniaturized AF 4 channel that has recently been developed to use microflow regime, with ESI-MS-MS in aqueous phase. HDL and LDL particles from blood sample were separated by sizes in aqueous solution in cAF 4 channel and the eluting lipoprotein particles were directly analyzed by direct ESI-MS-MS. By using on-line cAF 4 -ESI-MS-MS, lipoprotein particles can be desalted on-line during cAF 4 separation which enhances ionization of lipid molecules contained in lipoproteins during ESI-MS. Structural determinations of lipid molecules including phospholipids, lysophospholipids, cholesteryl esters, and triacylglycerols were carried out with in-source fragmentation during ESI followed by data dependent CID experiments.

PWe-071
11:10 – 12:20

Potential Biomarkers of Coronary Artery Disease by Flow Field-Flow Fractionation and Nanoflow LC-ESI-MS-MS

Seul Kee Byeon, Ju Yong Lee, Myeong Hee Moon

Yonsei University, Seoul, Korea

Keywords:
nLC-ESI-MS-MS, CAD, biomarkers, phospholipid, flow field-flow fractionation

Novel aspects:
Potential candidate biomarkers of coronary artery disease can be identified by profiling phospholilpids and lysopholipids from human plasma using nLC-ESI-MS-MS.

Abstract:
Ranking high in the leading causes of cardiovascular diseases, coronary artery disease (CAD) is commonly found well over the world, ranging from industrialized countries to less-developed countries. Although it has been generally diagnosed in people over 40 years-old, current trend in CAD is starting to be affiliated with younger generation of age under 20 as well. As a disease that can affect everyone regardless of sex and age, attention on CAD in an attempt to understand and explain the disease has been increasing high in medical and chemical fields. There are many reported risk factors of this disease including high blood pressure, lack of exercises, obesity, smoking, diabetes, high cholesterol, and high level of low-density lipoprotein (LDL) and low level of high-density lipoprotein (HDL) are highly related to pathogenesis of the diseases. Among different subclasses of lipids, studies on phospholipids have sparked an interest for the past decades because of their propensity to control various types of cellular responses. Numerous studies that have been published nowadays engage with lysophospholipids (LPL) , which are phospholipids (PL) with one fatty acid chain instead of two, and PLs because they are reported as biomarkers of human diseases such as breast, prostate, and ovarian cancer. In this study, LPLs and PLs from plasma of control group and patients with CAD were identified and compared in order to discover potential biomarkers of the disease.

First, HDL and LDL from human plasma were separated according to the particle size by using flow field-flow fractionation (FlFFF) . Among different types of FlFFF techniques, multiplexed hollow fiber flow field-flow fractionation (MxHF 5) was specifically chosen in order to inject a substantial volume of plasma sample at a time. The fractionated HDL and LDL were collected over time, and intact LPLs and PLs were simultaneously extracted while proteins were removed from these collections using a modified Folch method with methyl-tert-butyl ether (MTBE) and methanol. In order to identify as much LPLs and PLs as possible, including the species that existed in trace amount, nanoflow liquid chromatography electrospray ionization-tandem mass spectrometry (nLC-ESI-MS-MS) was employed so that LPLs and PLs could be identified in an order of hydrophobicity, regardless of their amount. A comprehensive analysis of LPLs and PLs from control and patients was performed both qualitatively and quantitatively, by nLC-ESI-MS-MS. In order to produce a highly-reliable and valid data, a total of ten plasma samples from each group were obtained and analyzed.

PWe-072
13:30 – 14:40

Profiling of the Oxidized Phospholipids in Human Lipoprotein by Nanoflow LC-ESI-MS-MS

Ju Yong Lee, Myeong Hee Moon

Yonsei University, Seoul, South Korea

Keywords:
nLC-ESI-MS-MS, oxidized LDL, Phospholipid, Lysophospholipid

Novel aspects:
Using collision induced dissociation patterns of the oxidized phospholipids obtained from nLC-ESI-MS-MS, phospholipids from oxidized LDL can be identified.

Abstract:
Metalloprotein hemoglobins (met-hemoglobin) refer to a complex when the charge of an iron ion from hemoglobin changes from +2 to +3 and function differently. They oxidize lipoprotein, apolipoproteins, and phospholipids to transport the lipid by blood vessel. According to recent studies, it is known that oxidized low-density lipoproteins (LDL) function differently from intact LDL and their particle sizes and characteristics as well. Moreover, as macrophages fail to get rid of oxidized LDLs, they travel via blood stream inside human body and end up causing severe inflammation in arteries, which can lead to one of most common heart disease, called Coronary Artery Disease (CAD). Despite the fact that oxidized LDLs play an important role in human body, not much studies have been conducted so far, and among a few number of studies that have been published, their investigation is primarily on oxidized phosphatidylcholine (PC) only. In this study, a systematic analysis of different classes of oxidized phospholipids (PLs) has been carried out to discover the detailed molecular patterns.

To examine the oxidation pattern of PLs, standard PLs (18 : 0/18 : 0-PC, 16 : 0/22 : 6-PC, 18 : 0/22 : 6-PG, and 18 : 0/22 : 6-PA) were utilized to make each vesicle first, and then the resulting vesicles were oxidized by adding 10 μM $CuSO_4$. The mixtures were stored at 37&#8451 ; for two different periods of 1 and 2 hours. In order to promote quenching the oxidation reaction, EDTA was added to the samples. For oxidation of LDL particles, $CuSO_4$ was mixed with LDL standard and stored for two hours. Nanoflow liquid chromatography electrospray ionization tandem-mass spectroscopy (nLC-ESI-MS-MS) was utilized to characterize oxidized PL molecules.

For PLs with saturated acyl chains, there was not much of a change observed. However, for PLs with poly unsaturated acyl chains, a variety of oxidation in acyl chains was observed regardless of head groups ; addition of hydroxyl group to fatty acids and production of different types of lysophospholipids (LPLs). Based on these results, intact LDL and oxidized LDL were analyzed by nLC-ESI-MS-MS under the same conditions and a total of 160 PL species (including oxidized PLs) were identified.

PWe-073
11:10 – 12:20

Discovery of lipid markers by LC-ESI/MS for early detection of low dose radiation exposure

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National Health Research Institutes, Zhunan, Taiwan

Keywords:
lipids, radiation, biological dosimetry, sphingomyelin, ceramide

Novel aspects:
lipid molecules of cell membrane act as biological dosimetry for low dose exposure of radiation

Abstract:
The Japanese nuclear accident in 2011 has awakened us to pay much attention to nuclear safety and health risk for the exposure of radiation. In conventional scope, alteration of genomic DNA was believed the primary and unique molecular marker owing to its critical role in delivery of genetic information. Radiation undoubtedly influences cell membrane together with DNA ; and we try to use HPLC-ESI/MS for rapid discovery of cell membrane-related markers. The specific aim is to clarify the chemical differences of lipids on cell membrane prior to chromosome aberration. We anticipate to (1) explore early detection marker for radiation exposure of citizens and (2) advance understanding of lipid damage mediated apoptosis for scientists in the field of radiobiology and radiotherapy. Human glioblastoma U87-MG cells and mouse colon carcinoma CT26 cells were irradiated with X-ray for 0.5 and 5.0 Gy of doses in comparison to untreated cells. Twenty-four hours after X-ray irradiation, cells were lysed with isopropyl alcohol (IPA) followed by centrifugation at 12500 g for 30 min at 10oC. The phospholipids' extracts were harvested using solid phase extraction (Ostro(R) 96-well plate, Waters) for further analysis by HPLC (Agilent 1100) conjugated with high resolution ESI/MS (microTOF II focus, Bruker Daltonics) . Chromatograms and mass spectra were displayed and reconstructed using Compass DataAnalysis (version 4.0, Bruker Daltonics) . The identification of molecular formula was based on the exact mass of monoisotopic peak with the tolerance of 5 mDa. Sphingomyelin (d18 : 1/16 : 0) eluted around 27 min with 703.57 m/z of protonated molecular ion was significantly eliminated by the increase of X-ray dose, which directly correlated with the increase of signals with 538.52 m/z, eluted at 3.4 min and assigned as ceramide (d18 : 1/16 : 0) . Several phosphatidylethanolamines (PEs) and phosphatidylglycerols (PGs) were found and positively correlated with increasing dose of X-ray radiation, whereas phosphatidylcholines (PCs) represented no significant change. Co-stained with propidium iodide (PI) and YOPRO-1 demonstrated the biological property of cell membrane did change after the treatment of radiation. We therefore suggest the breakdown of sphingomyelin to ceramide could be a potential diagnostic biomarker for low dose exposure (<0.5Gy) of radiation.

PWe-074
13:30 – 14:40

Characterization of moquito cuticular lipids for age, species, and sex discrimination by MALDI-IT-TOF-MS

Kevin A Schug[1], Sheheli Islam[1], Jaroslaw Krzywinski[2], Seoung Bum Kim[3]

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Keywords:
mosquito, MALDI, cuticular lipids, tandem mass spectrometry

Novel aspects:
MALDI-IT-TOF-MS is used to identify specific cuticular lipid components, which can be used to differentiate species, sex, age, and mating status of malaria-carrying mosquitoes

Abstract:
Malaria is an insidious disease. Millions of people are affected, hospitalized, or killed annually as a consequence of infection. The malaria parasite is tranferred to humans through the bites older female *Anopheles gambiae* mosquitoes. Currently, treatment strategies for malaria are limited, and much effort is given to risk assessment and abatement. To assess the risk of infection for a given community, it would be ideal to understand the proportion of mosquito population that is capable of conferring the parasite. Previously, our group has published a MALDI-TOF-MS method (*Anal. Chim. Acta***2011**, *706*, 157-163) , which, based on mass spectral fingerprints generated following chloroform extraction of exoskeletal compounds, can be used to differentiate *A. gambiae* mosquitoes based on their age, sex, and mating status. Cuticular lipids and hydrocarbons are well known to help prevent dessication of insects and contribute to mate recognition. Signals are generated through the combined use of an acenaphthene matrix and a $AgNO_3$ cationization reagent. Though useful, relatively little information could be obtained regarding the identity of signals which differentiate between classes. More recently, we have obtained a Shimadzu Resonance MALDI-ion trap-time of flight-MS instrument. Capable of isolation, multi-stage fragmentation, and high resolution measurement of MALDI signals, we have now been able to pursue identification of important lipids compounds obtained from the extract. Our preliminary results show that a mix of hydrocarbons (< 500 Da) and lipids (500 - 1200 Da) are extracted and contribute to the fingerprint. Lipids are believed to include different chain length variants of ceramides and sphingomyelins. Work is ongoing to unequivocally identify these species. Additionally, based on the increased sensitivity and resolution of the new instrument, we are able to perform extracts of single mosquitoes, whereas three mosquitoes were necessary with the previous instrument. Besides discrimination of attributes within just the *A. gambiae* species, we also will show efforts to discriminate between different mosquito species. These developments bring higher relevance to potential risk assessment strategies, and the further knowledge of specific cuticular hydrocarbons and lipids should help researchers develop more effective risk abatement and treatment strategies.

PWe-075
11:10 – 12:20

Liquid extraction surface analysis for local profile of lipid molecular species on mammalian tissue slices

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Keywords:
phospholipid, surface-analysis, direct-extraction, tissues, localization.

Novel aspects:
Sensitive and practical analysis of localized position of tissue slices were effectively obtained by direct liquid extraction from tissue slice surface.

Abstract:
Liquid extraction surface analysis (LESA) was applied to obtain local profiles of lipid molecular species on individual specific parts of mammalian tissue slices from brain, heart or kidney. Also LESA was applied for some transgenic and disease model animals. Obtained mass spectometric data were automatically merged qualitatively and quantitatively by new version of our search engine "Lipid Search " (http : //lipidseach.hs.chubu.ac.jp) . Several characteristic differences in profiles of individual phospholipid molecular species were further analyzed by principal component analysis (PCA) and discrimination analysis.
Method : Frozen mouse or rat tissues such as brain, heart or kidney were cut into slices at 15μm thickness by Leica CM1950 cryostat. Resulting slices were subjected to LESA-NanoMate (Advion) analysis with 4000Qtrap (ABSciex) or Orbitrap (Themo Fisher) . Chloroform/methanol (1 : 2) containing 5mM ammonium formate was used as extracting and ionization solvent. Solution volume of 1.6μL was used for each analysis area (1 - 2 mm diameter) . MS analysis was operated with flow rate at 200nL/min and with using the analytical mode such as EMS, precursor ion scanning and neutral loss scanning both in positive and negative ion mode by 4000Qtrap. Also more precise and high resolution MS data were obtained by Orbitrap.
Result : Detection efficiency by nanoESI by Triversa NanoMate combination with LESA system seems to be very sensitive, because of their high recovery by direct surface extraction. By LESA system most of major lipid molecular species were effectively extracted and detected from specific localized parts at around 1 - 2 mm diameter of several mammalian tissue slice surface. We could obtained characteristic mass profiles of most of major phospholipid molecular species from several organs, such as brain, kidney and heart from mouse or rat.
As the results of LESA, it was revealed that molecular species such as 18 : 0/18 : 1 of phosphatidylcholine (PC) , phosphatidylethanolamine (PE) and phoshatidylserine (PS) and are rich in myelin layer of cerebellumn, and reticular formation, and relatively low in hypocampus, cerebral cortex, molecular and granular layer of cerebellum both in mouse and rat brain. Detection efficiency of LESA with nano-ESI by NanoMate is revealed to be very sensitive. And the sensitivity is almost compatible to MALDI-TOF, but detectable molecular species were more rich in nano-ESI by LESA than that of MALDI, especially for PE or PS molecular species. Because of low space resolution efficiency of LESA, the narrow area less than 1 mm diameter such as callosum was difficult to be analyzed as a specific limited area. Characteristic molecular profiles in localization of several major phospholipid molecular species from mouse brain obtained by LESA were almost identical to those obtained by laser micro dissection (LMD) combination with nanoESI-MS. The profiles in molecular species of glycosphingolipids such as gangliosides and sulfatides, and those of triglycerides were also effectively obtained with same extraction condition.
Local characterization of phospholipid molecular species in cortex and medulla from mouse and rat kidney was also effectively obtained. Contents of arachidonic acid containing PC molecular species were richer in medulla than cortex. Concerning to phospholipids of kidney, content of docosahexsaenoic acid (DHA) containing molecular species were much higher in mouse than rat.
Conclusion : Liquid extraction surface analysis (LESA) combination with NanoMate is revealed to be a practical and sensitive method for the localization analysis of lipid molecular species in mammalian tissue slices at 1 - 2 mm resolution. We effectively obtained 100-200molecular species of individual classes in phospholipids, triglycerides and glycosphongolipids such as gangliosides and sulfatides. Characteristic features in each different domain in several mammalian tissues were effectively observed.

PWe-076 Comprehensive Standardized Methods of Lipid Profiling

13:30 – 14:40

Toshiyuki Yamazaki, Noriko Fueki, Nembai Tomoko, Karasawa Kaoru

AB SCIEX, Tokyo, Japan

Keywords:
Lipidomics, Metabolomics, Q-TOF, Accurate Mass, Library

Novel aspects:
This presentation discribes two comprehensive standardized method of Lipid Profiling.These methods are aplicable for phospholipids and the other lipids.

Abstract:

Novel Aspect

We present comprehensive standardized methods of lipid profiling. These methods are applicable for phospholipids and the other lipids.

Introduction

Phospholipids are very complex mixtures of closely related compounds. Shotgun lipidomics is a powerful approach for the analysis of lipids from extracts of biological tissues by direct infusion. On the other hand, a reverse-phase high performance liquid chromatography method is capable of separating isomers of lysophospholipids differing in the location of the aliphatic chain (sn-1 orsn-2 position) and the position (Δ6 or Δ9).

AB SCIEX Triple TOF™ 5600 System delivering high-resolution for high-speed chromatography - up to 100spectra in a second or 50 spectra in a cycle while Information Dependent Acquisition (IDA). In the shotgun lipidomics, the mass selective quadrupole, Q1 is set to step 1 Da increments across the mass range, selecting lipid precursor ions at unit resolution. Isolated precursor ions are transmitted through the LINAC® collision cell for complete CID fragmentation and all fragment ions are recorded as high resolution TOF spectra. Data processing was done with PeakView® Software. And lipid identification and quantitation was done with LipidView™ Software.

Materials & Methods

< Samples for methods development >
Egg yolk extracted with Bligh-Dyer procedure using about 10mg of egg yolk and redissolved in IPA.

< MSMSALL (infusion) >
Continuous-Infusion
Mass Spectrometry : AB SCIEX Triple TOF™ 5600 System
Accumulation time : TOFMS 1 or 10sec,Product Ion Scan 0.1 or 1 sec
Mass range : m/z 200 to 950 Da
Acquisition time : 1.3min or 13min
Collision Energy : 40V
Collision Energy Spread : 20V

< Liquid Chromatography (MS screening and MS/MS Library Search) >
HPLC Column : ACQUITY UPLC HSS T3 (2.1×50mm)
Solvent A : 5 mM ammonium formate in H_2O, B : 5 mM ammonium formate in H_2O IPA
Mass Spectrometry : AB SCIEX Triple TOF™ 5600 System
TOFMS 0.15sec, Product Ion Scan 0.05sec (20 times / cycle)
Mass range : TOFMS : m/z 200&#65374 ; 950 Da, Product Ion : m/z 150&#65374 ; 950 Da
Acquisition time : 25 min
Collision Energy 40V, Collision Energy Spread20V

Preliminary Data

< MSMSALL (infusion) >

MS/MS spectra were acquired from 200 to 950m/z accumulated for 100 ms each and the total cycle time for the experiment were 1.3 min per polarity. For minor component, 1 second accumulation time was used instead. MS/MS ALL workflow had shown that egg yolk has a combination of 7 major different acyl ions those were also detected providing molecular species quantification in a peak area response. Peaks of the acyl anion of FA 18：0 (m/z 283.2643) and the product (m/z 283.2431) of CO_2 loss from the acyl anion of FA 22：6 were distinguished with accurate mass in egg yolk.

< Liquid Chromatography >

Isomeric lysoPCs were distinguished based on retention time and the peak intensity ratio of product ions, and 7 pairs of lysoPC isomers were identified in egg yolk. XIC Manager, a function of PeakView(R) Software, can be used for targeted and non-targeted processing of high resolution MS and MS/MS data allowing for screening and identification with the highest confidence based on retention time, accurate mass molecular ion, isotopic pattern, and automatic MS/MS library searching. We are going to establish automatic identification and quantitative processing workflow using XIC Manager.

PWe-077
11:10 – 12:20

The utility of acetonitrile-related adducts formed in APCI sources for localization of double bonds in lipids

Josef Cvacka[1], Vladimir Vrkoslav[1], Eva Hakova[2], Petra Horka[1,2]

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Keywords:
lipids, structure elucidation, double bonds, HPLC/MS, gas phase reaction

Novel aspects:
Broadening the application area of recently discovered method for localization of double bonds for new lipid classes. Investigation of the gas-phase reaction mechanism.

Abstract:
Most lipids contain long chains derived from fatty acids, alcohols, or other long-chain compounds. The presence of double bonds, their number, relative position and *cis-/trans*-isomerism strongly affects physical, chemical and biochemical properties of lipids, which has important consequences on their functions in living organisms. Localization of double bonds in unsaturated long-chain compounds is a classical analytical problem, especially challenging when the analytes are present in complex mixtures and low quantities. There are numerous methods allowing for determination of double bond positions using mass spectrometry, but they are mostly based on electron ionization (EI) . The applications of EI are considerably limited for sufficiently volatile and thermally stable compounds. Most lipids are not directly amenable to GC/EI-MS and must by hydrolyzed prior to their analysis ; an important piece of information on their structures is lost. Therefore, methods making localization of double bonds possible in the intact lipids are highly desirable.
We have recently shown that gas phase reactions of acetonitrile taking place in APCI sources can be utilized for localization of double bonds in unsaturated esters. Wax esters and methyl esters provided $[M+C_3H_5N]^{+\cdot}$ adducts. When fragmented by CID, pairs of highly abundant ions allowing for unambiguous localization of double bonds were generated. In this work we investigated unsaturated lipids from various classes to learn if they undergo similar type of reactions. Triacylglycerols with diverse FA chains were prepared by a randomization reaction optimized for a small scale. It was found that all investigated unsaturated lipids including long-chain compounds with various functional groups, diol diesters, acylglycerols and other lipids formed the above mentioned adducts and provided expected diagnostic fragments suitable for localization of double bonds. Therefore, the method can be generally used for the lipids amenable to APCI. As the reactant (acetonitrile) is a common solvent in HPLC, double bonds can be conveniently localized in the molecular species separated by liquid chromatography, typically in non-aqueous reversed phase systems. The influence of the arrangement of double bonds in polyunsaturated chains on the adduct formation and the appearance of the MS/MS spectra was studied using FAMEs with isolated, methylene-interrupted, conjugated and cumulated double bonds. The mechanisms behind formation of the $[M+C_3H_5N]^{+\cdot}$ adducts was investigated using stable isotope labeled acetonitriles and higher nitriles (propionitrile, butyronitrile) .
Financial support from the Czech Science Foundation GACR (Project No. 203/09/0139) and the Academy of Sciences of the Czech Republic (Project RVO: 61388963) is acknowledged.

PWe-078
13:30 – 14:40

New mass spectrometric methods for structural characterization of hydrocarbons

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Keywords:
Hydrocarbons, structural characterization, double bond position, branching position

Novel aspects:
New APCI and MALDI mass spectrometric methods for determination of double bond (s) and branching position (s) in hydrocarbons based on fragmentation of the molecular adduct.

Abstract:
Gas chromatography/electron ionization mass spectrometry (GC/EI-MS) is the most often used method for analysis of hydrocarbons. EI spectra do not provide important information about position of double bonds. Characterization of branching positions is some times unclear. Double bond of hydrocarbons could be localized from fragments of acetonitrile related adduct after chemical ionization (CI) , or from fragments of EI ion source after suitable derivatization. However, derivatization is time consuming. Moreover, themolecular weight of detected compounds is limited by the functional range of the method, i.e. the technique is limited to hydrocarbons with up to about 50 carbon atoms. Unsaturated hydrocarbons with even less carbons are rapidly decomposed or rearranged due to high temperatures in the GC injector and/or column. Obviously, principally different analytical methods are required to broaden the range of molecular weights and thus to allow analysis of hydrocarbons with considerably longer aliphatic chains. The main goal of this project is to develop new methods for structural characterization of long-chain aliphatic hydrocarbons. The methods are based on MS/MS of molecular adducts obtained by MALDI and APCI technique.
It is known that matrices containing lithium or silver cations work the best for MALDI of hydrocarbons. Hydrocarbons are detected as lithium or silver molecular adducts ($[M+Li]^+$, $[M+Ag]^+$) . MALDI-MS allows determination of molecular weights, which is quite important, but there are no structure-related fragments in the MS spectra. MS/MS spectra of long-chain hydrocarbons give fragments, which can by use for identification of position of branching point (s) of alkyl chain. We use LIFT technique (Ultraflextreme, Bruker, Bremen, Germany) to obtain MS/MS spectra of $[M+Ag]^+$and $[M+Li]^+$ of various hydrocarbons like Apolane-87 (22,24-diethyl-19,29-dioctadecylheptatetracontane) or squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene) . The technique provided fragments, which localized positions of branching.
The presence of double bonds and their number, position and *cis-/trans-* isomerism strongly affects physico-chemical properties of hydrocarbons and their functions in biological systems. Acetonitrile-related adducts $[M+55]^{+\cdot}$ formed in the APCI source for localization of double bond position (s) in wax esters were described previously. $[M+55]^{+\cdot}$ adducts in APCI ion source were also observed, when unsaturated hydrocarbons were mixed with acetonitrile. Molecular composition of adduct was determined by accurate mass measurement as $[M+C_3H_5N]^{+\cdot}$. The CID MS/MS of $[M+C_3H_5N]^{+\cdot}$ yielded fragments allowing the localization of double bond (s) in the hydrocarbon chain. Fragmentation corresponds to cleavage next to double bond.

Financial support from the Czech Science Foundation (Project No. P206/12/1093) and the Academy of Sciences of the Czech Republic (RVO:61388963) is herewith acknowledged with appreciation.

PWe-079
11:10 – 12:20

Comparison of triacylglycerols in commercial DHA supplements utilizing MALDI-TOF-TOF tandem MS with spiral ion trajectory

Ayumi Kubo, Yoshiyuki Itoh, Masahiro Hashimoto, Jun Tamura

JEOL Ltd., Tokyo, Japan

Keywords:
high energy CID, high precursor ion selectivity, MS/MS, MALDI,Triacylglycerol

Novel aspects:
Structural analysis of triacylglycerols in comercial DHA supplement using MALDI-TOF-TOF tandem MS without chromatographic separation and derivatization.

Abstract:

Introduction

In recent years, docosahexaenoic acid (DHA) supplements are frequently promoted as functional foods because DHA is believed to be helpful to people with heart disease, for premature infants, and to support healthy brain development. Main constituent of many of the DHA supplements is fish oil, or triacylglycerol derived from fish. It has been recognized that biological activities of triacylglycerols depend not only on their overall fatty acids composition but also molecular species composition and position-specific (or "regiospecific ") distribution of fatty acids. Such molecular and regiospecific analysis, however, has not been performed very often because of the tedious analytical procedures required. We analyzed triacylglycerols of DHA supplements using MALDI-TOF-TOF tandem MS without chromatographic separation and derivatization.

Methods

All of the DHA supplements were purchased at the local drugstore. They were almost in the form of soft gelatin capsule. The contents of the capsules were dissolved into tetrahydrofuran (THF) at the concentration of 10 mg/mL. The 2,5-dihydroxybenzoic acid DHB matrix solution was prepared at the concentration of 20 mg/mL in THF. The cationization agent solution was prepared by dissolving NaTFA at the concentration of 10 mg/mL in THF. For final sample preparation, one of the analyte solutions, matrix solution, and cationzed agent solution were mixed at 1 : 1 : 2 volume ratio and 1 μL of the mixture was applied onto the stainless steel target plate. A JMS-S3000 "SpiralTOF " MALDI-TOF-TOF tandem MS (JEOL Ltd., Tokyo, Japan) was employed for all the analyses.

Preliminary Data

On the mass spectra of all the supplements analyzed, peaks that can be attributed to sodium ion adduct of triacylglycerol molecules containing polyunsaturated fatty acids (PUFA) , such as DHA and eicosapentaenoic acid (EPA) , are observed in mass range over 900. The observed mass resolution of those peaks was between 50000 and 60000 at FWHM. The difference between observed m/z value and theoretical m/z value for each of the triacylglycerol peak was less than 10 ppm by using sodiated poly (propylene glycol) as the external mass calibration standard. By comparing mass spectra from various DHA supplements, it was found that triacylglycerols contained in the supplements vary significantly. In addition, the chemical structures of several triacylglycerols were investigated by acquiring the product ion mass spectra of monoisotopicaly selected precursor ions. As a result, we found triacylglycerols containing DHA with the double bonds at w-3,6,9,12,15,18 positions. We also confirmed the presence of several regioisomers of triacylglycerols.

PWe-080
13:30 – 14:40

Analysis of oxidized lipid derived volatiles by Gas Chromatography/Mass Spectrometry

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Keywords:
aldehyde, lipid peroxidation, In-tube extraction

Novel aspects:
Exhaustive volatile compounds analysis allowed us to understand oxidized lipid production mechanism and might lead to be able to analyze diseases by malti-marker profiling.

Abstract:
Lipid, which is main component of cell membrane, might provide harmful peroxidation derivatives in the particular condition under oxidative stress. During the oxidative reaction, polyunsaturated fatty acid motifs are oxidized at a double bond carbon to produce oxidized lipids. Volatile compounds including short-chain aldehydes are also released from oxidized lipids. However, the production of many kinds of volatile compounds depends on various carbon chain or different unsaturated degree fatty acids and the production of these compounds is very trace. In addition, it is known that these compounds react with biological metabolites and generate different compounds. Therefore, an advanced technique that enables exhaustive analysis of these compounds is required. In a previous study, it was reported that specific odors were released from individual diseases and these odors were generated differently in each diseases. Although there have been reports on the analysis of particular short chain aldehydes, an exhaustive analysis for short chain aldehydes in blood or plasma samples has not been reported. In this study, Head-Space Solid Phase Micro Extraction (HS-SPME) was selected as a method to extract volatile compounds. However, it was difficult to perform detailed analysis due to the presence of some foreign substances in biological samples thus negatively affects the sensitivity. Therefore, we attempt to develop the In-tube extraction (ITEX) method and gas chromatography/mass spectrometry to analyze various volatile compounds produced by oxidizing lipids.

It is possible that there are many unreported volatile compounds exist in samples in addition to aldehydes. At first, oxidized polyunsaturated lipid samples *in vitro* were prepared to construct the analytical system for volatile compounds derived from oxidized lipids. We oxidized phosphatidylcholine (PC) samples by oxidant, 2,2' - azobis (2-aminopropane) dihydrochloride (AAPH). As a result, aldehydes or hydroxyl compounds as well as cyclic volatile compounds; 2-pentylfuran, and compounds with a number of double bonds; 2,4-alkanedienal, were identified from these oxidized lipid samples. Furthermore, we applied the method using mouse plasma as a biological sample. Aldehyde and hydroxyl compounds were identified in mouse plasma. In addition, 2-pentylfuran was also identified from these samples that are similar with previous volatile oxidized lipid samples.

For the future, we will continue to analyze various volatile oxidized lipid samples to construct volatile compounds library for real biological sample analysis and apply the method for analysis of biological samples.

PWe-081
11:10 – 12:20

Structural analysis of triacylglycerols using desorption electrospray ionization mass spectrometry with precursor ion scanning and neutral loss scanning

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Keywords:
Desorption electrospray ionization, triple quadrupole mass spectrometer, triacylglycerol, lipid

Novel aspects:
DESI, coupled with the triple quadrupole mass spectrometer, can provide detailed structural information and it is an effective technique for the structural analysis for lipid.

Abstract:
Desorption electrospray ionization (DESI) is an ionization method that enables the direct and rapid analysis of biological samples including whole organs. This ionization technique has been applied to lipidomics in combination with some mass spectrometers, especially ion trap mass spectrometers. Nevertheless, few reports have described DESI-MS analysis using a triple quadrupole mass spectrometer. This spectrometer, a powerful tool for structural analysis, can perform three kinds of MS/MS experiments, product ion scanning, precursor ion scanning, and neutral loss scanning.

This work demonstrates the availability of DESI-MS for structural analysis of lipid on surfaces using triple quadrupole mass spectrometer without extraction. This analysis was also extended to the direct analysis of biological samples.

Commercially available edible meat, beef, pork, and salmon were used as samples directly without preparation. Samples were analyzed using positive or negative ionization mode. Product ion scanning was performed to confirm the structure. Neutral loss scanning and precursor ion scanning were also performed for structural analysis. Neutral loss scanning of 273, 271, 301, 299, and 297 Da were used. To compare lipid profiles analyzed for ESI and DESI, the extracted lipids were analyzed using flow injection. Lipids were extracted from meat according to solid-phase extraction and Bligh & Dyer method.

In this system, fatty acids (negative mode) , monoacylglycerol, diacylglycerol, and phosphatidylcholine (positive mode) were detected. MG and DG ions were detected in higher sensitivity than ESI-MS was. These molecules were ionized more easily than TG in this ionization method. Moreover, the intensity of TG molecular species consisted of numerous double bonds. Long acyl chains were few in this system. These results suggest that the efficiency of ionization depended on the lipid class. The abundant fatty acids of beef were saturated fatty acid, palmitic acid, and stearic acid. Those of pork were mono-unsaturated fatty acids, palmitoleic acid, and oleic acid. The results agree with those of previous reports. Product ion scanning and precursor ion scanning gave detailed information related to each TG structure. The TGs with a specific fatty acids were detected with neutral loss scanning in positive mode. These results show the specific TG profiles for each edible meat. However, in this condition, polyunsaturated and TG-containing polyunsaturated fatty acids, EPA and DHA, were not detected clearly. These results support that the conditions for neutral loss scanning in DESI-MS analysis depended on the chemical structures such as the number of carbon and/or double bonds in the molecule. It is necessary for quantitative analysis and profiling analysis to optimize the detailed analytical conditions for fatty-acid-containing long acyl chains.

DESI, coupled with the triple quadrupole mass spectrometer, can provide detailed structural information. It is an effective technique for the structural analysis of lipid. These results show that DESI-MS is a useful approach for the lipid analysis on surface providing detailed structural information. Moreover this simple and quick analysis system is expected to be valuable for the direct structural analysis of lipids.

PWe-082 Multidimensional lipidomics for untargeted and targeted analyses

13:30 – 14:40

Giuseppe Astarita[2], Marc V Gorenstein[2], Andrew Baker[2], Mark A Ritchie[1], John P Shockcor[2]

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Keywords:
Lipids, HILIC UPLC, IMS ToF, MSE

Novel aspects:
Class based separation of lipids provides a diagnostic separation which is resolved and defined using IMS-ToF, allowing highly complex samples to be screened with high descrimination

Abstract:
One of the main challenges for a global lipid analysis lipidomics is the separation of the wide array of lipid species present in biological samples. The ability to perform multi-dimensional separations in one injection prior to MS analysis could improve our ability to map and measure complex lipid mixtures. Here, we present a complete workflow for the extraction, separation, identification and quantification of lipid species in complex matrices.

We first used an off-line enrichment system for the extraction of lipid species from biological samples. Next, we analyzed lipid extracts using Ultra Performance LC (UPLC) -MS giving class distinct separation of the lipids in a 10 minute gradient run. An ion mobility-TOF system was used to discriminate lipids classes in their constituents based on the different cross collision sections. Lipid ions with different degree of unsaturation and acyl length migrate with characteristic mobility times due to their unique interactions with the nitrogen gas in the ion mobility cell. Using this novel technological approach, we applied a peak detection algorithm to generate molecular maps of lipids present in various animal tissues and serum, which could facilitate comparative lipidomic analysis. To gain more structural information, lipids were analyzed using parallel low and elevated collision energy (MS^E) to acquire both precursor and product ion information in a single chromatographic run, increasing the specificity of identification. The database of mass transitions was used to program tandem quadrupole MRM method and automated processing methods for the high throughput quantification of selected lipid species in biological samples.

In conclusion, the combination of UPLC with ion mobility-MS^E delivers a multi-dimensional characterization of complex biological mixtures, enhancing the lipidomic profiling and quantification.

PWe-083

11:10 – 12:20

Development of comprehensive identification and quantification of polyunsaturated fatty acids and eicosanoids in human plasma using LC-MS/MS

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Keywords:
Eicosanoids, PUFAs, Human plasma, Comprehensive identification and quantification, LC-MS/MS

Novel aspects:
Development of selectivity and sensitivity 3 several comprehensive quantification method (Free fatty acids, total fatty acids, about 100 of eicosanoids) using LC-MS/MS to be able to apply in clinical study.

Abstract:
Polyunsaturated fatty acids (PUFAs) that are taken by human, these PUFAs are form to membrane phospholipids to composition cell membranes. These membrane phospholipids are hydrolyzed by phospholipase A_2 (PLA_2) then break down phospholipids into fatty acids and cyclooxygenase (COX) or lipoxygenase (LOX) catalyzes converse the fatty acids to eicosanoids. Eicosanoids are considering to be signaling compound act as autocrine or paracrine. Recently strong relationship with eicosanoids and various diseases, such as arteriosclerosis, rheumatism and hyperalgesia are demonstrated by *in vitro* or using several model mice and have revealed the mechanism of the diseases gradually in numerous studies. These researches about eicosanoids and relationship with various diseases are increase at an accelerated rate. However, there are few reports about eicosanoids analysis in clinical studies. These reasons are the body tissue possible to taken from alive human are confined like blood, urine or saliva. Especially, the blood is one of the tissue of accurately reflect the physical health. However the blood is complex matrix and concentration of the eicosanoids in blood is extremely low (most of the concentrations of eicosanoids are about pg/mL or fg/mL level) , therefore high selectivity and sensitivity method is required. Furthermore, after blood is taken, PUFAs are metabolizing to eicosanoids *ex vivo* because of the COX in platelet. Therefore, it's difficult to measure the eicosanoids concentration stably in clinical study. Therefore we validated the blood collection protocol from human and also developed the 3 several comprehensive quantification method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to be able to apply in clinical study. First methodology is quantitative total fatty acids (Arachidonic acid, Eicosapentaenoic acid, Docosahexaenoic acid) , second is free fatty acid and third is about 100 compounds of eicosanoids were targeted. Total fatty acids, free fatty acids were extracted from 20 uL of human plasma by liquid-liquid extraction respectively. And about 100 compounds of eicosanoids were extracted from 1 mL of human plasma by solid phase extraction. The extracted solution was injected to LC-MS/MS and all compounds were performed in negative ion electrospray ionization scheduled SRM mode. For the calibration curve to quantitate the concentration in plasma sample, we used PBS for the surrogate matrix. Finally, we developed the robust method for comprehensive identification and quantification of polyunsaturated fatty acids and eicosanoids and we figure out the baseline level from the data of healthy human subjects. Our developed method will be characterized the subjects andefficient platform in stage at evaluation of drug efficacy in clinical study.

PWe-084 Chiral HPLC/MS Analysis of Triacyglycerol Enantiomers and Regioisomers

13:30 – 14:40

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Keywords:
Lipidomics, HPLC/MS, chiral HPLC, triacylglycerol, enantiomer

Novel aspects:
Analysis of wide range of TG enantiomers and regioisomers in one analysis using chiral HPLC/MS method.

Abstract:
Triacylglycerols (TGs) are important components of human diet used as a source of energy, fatty acids (including essential ones) , fat soluble vitamins and other nonpolar compounds. The variety of TG species is enormous due to a high number of fatty acids present in natural samples differing in acyl chain lengths, their position on the glycerol skeleton (region isomers, enantiomers) and number, position (s) and *cis-/trans-* configuration of double bonds (DBs) , all having great differences in their biological and nutritional properties. The detailed characterization of fatty acid composition including the determination of individual isomers is important from the nutrition point of view due to their different biological activity. The stereospecific analysis of individual TGs represents a challenging task in the lipidomics due to different bioavailability of fatty acids on the glycerole skeleton in the stereospecific environment of enzymes in the human body. We have optimized a chiral HPLC/MS analysis for the separation of TG isomers using cellulose-based chiral HPLC column in the normal-phase mode. Under optimized chromatographic conditions, both regioisomers and enantiomers are separated in one analysis. The retention behavior of TGs is governed mainly by the number of DBs, *i.e.*, retention times of TGs increase with increasing number of DBs. At least partial separation of TGs based on acyl chain lengths or position and configuration of DBs is also achieved using optimized chiral HPLC method. TGs are identified based on their positive-ion atmospheric pressure chemical ionization (APCI) mass spectra providing protonated molecules and high relative abundance of fragment ions formed by the neutral loss of fatty acid from the glycerol skeleton. The comparison of relative abundances of these fragment ions is used for the differentiation of individual regioisomers, because the loss of fatty acid from sn-2 position is less preferred, which results in a fragment ion with lower relative abundance in comparison to *sn-*1 and *sn-*3 positions. No visible differences are observed in APCI mass spectra of enantiomers identified based on retention times of identical standards. We have developed a method for the synthesis of TG enantiomers with wide range of fatty acids commonly present in natural samples (*i.e.*, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids) . The retention behavior of TGs isomers is also studied using mixtures of TG isomers prepared by the randomization reaction of selected monoacyl TG standards providing mixtures of all isomers in an equal amount. We have demonstrated the utilization of developed chiral HPLC/MS method for the separation of complex natural TG mixtures of plant oils or human plasma samples.

This work was supported by the project 203/09/0139 sponsored by the Czech Science Foundation.

PWe-085
11:10 – 12:20

Structural analysis of oxidized triacylglycerols by using MALDI-SpiralTOF-TOF tandem mass spectrometer with high precursor ion selectivity and high-energy CID

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Keywords:
MALDI, MS/MS, High-energy CID, High precursor ion selectivity, Oxidized triacylglycerols

Novel aspects:
Direct structural analysis of thermally oxidized triacylglycerols by using the MALDI-TOF-TOF tandem mass spectrometer

Abstract:
Triacylglycerols are lipids that consist of a glycerol moiety with each hydroxyl group esterified to a fatty acid. Almost all fats and oils produced by animals and plants contain these compounds as a main ingredient. Additionally, it is well known that triacylglycerol, which has an unsaturated fatty acid moiety, is oxidized by air over time. Therefore, it is important to know the kinds of fatty acids and their position on the glycerol molecule in order for complete structural analysis of a particular oxidized/non-oxidized triacylglycerol. A tandem mass spectrometer with high-energy collision induced dissociation (HE-CID) provides a suitable method for obtaining structural information of triacylglycerol such as the positions of double-bonds, branching, and hydroxylation by means of charge-remote fragmentation. [1] The MALDI-TOF/TOF tandem mass spectrometer (MS) used in this work combines a SpiralTOF for the first TOFMS with an offset parabolic reflectron for the second TOFMS. [2] This TOF/TOF configuration offers several good performance characteristics for structural analysis of triacylglycerols in that it can monoisotopically select precursor ions, eliminate post source decay (PSD) ions and perform HE-CID at 20keV. [3]
In this work, we report the structural analysis of thermally oxidized triacylglycerols by HE-CID using the MALDI-SpiralTOF-TOF tandem MS.
As a starting point, triolein was used as a standard sample. Two triolein samples one as a pure sample, the other mixed with the antioxidant tocopherol (vitamin E) were oxidized in an oven set to 160 degrees Celsius in order to accelerate the oxidation process. Afterwards, each sample was analyzed by MALDI-SpiralTOF-TOF tandem MS.
Accuracy of all observed ions was within 5 ppm with external calibration. The results showed that the presence of the antioxidant was very effective in minimizing the oxidation of triolein. Additionally, the oxidized sample measured by HE-CID produced MS/MS spectra that contained enough information to determine the position of not only oxidization but also the double-bonds in the fatty acid moiety.
Next, commercial fats and oils like olive oil and margarine were also put into the oven at 160 degrees Celsius. These samples were measured at various times during the heating process to observe the changes in the oxidization by MALDI-SpiralTOF MS. Similarly, the structure of each oxidized triacylglycerol was determined using MALDI-SpiralTOF-TOF tandem MS.

PWe-086
13:30 – 14:40

Development of a high-throughput lipid profiling method by using a quadrupole orbitrap mass spectrometer and an automated lipid identification software

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Keywords:
lipidomics, high-throughput profiling, supercritical fluid chromatography, quadrupole orbitrap mass spectrometer, automated identification system for lipidome

Novel aspects:
We developed a high-throughput workflow for exhaustive lipid profiling by using Q Exactive and Lipid Search.

Abstract:
Lipids are the major components of biological membranes and energy storage substances, and they might function as signal transducers in some occasion. To prove the universality of the important contribution of lipids related compounds, the comprehensive analysis of lipid metabolism would be essential. Recently, the study of lipidomics, i.e., metabolomics for lipids, has been encouraged. There are a large number of lipid molecular species because of the diversity of polar head groups and hydrophobic fatty acid chains. For example, phospholipids have a hydrophilic head and one or two fatty acid chains, and triacylglycerols have three fatty acid chains. Thus, there are a variety of chemical structures and polarities in lipid molecular species. In mass spectrometry, certain molecular species show high sensitivity in the positive-ion mode, whereas others show high sensitivity in the negative-ion mode. Therefore, it is difficult to simultaneously analyze diverse lipid molecular species showing high sensitivity either in the positive-ion mode or in the negative-ion mode. In data processing, considerable time and effort is spent in manually identifying and listing all the molecular species in a biological sample. This is because it is necessary to confirm the multiple fragment ions of the polar head group and the fatty acid chains in order to identify the chemical structures of lipid molecular species.

In order to solve these problems, we developed a useful workflow using a quadrupole orbitrap mass spectrometer, which can perform high-resolution full scanning and product ion scanning in both the positive-ion mode and the negative-ion mode in practical cycle time, and an automated lipid identification system. The target lipids in this study are phospholipids, triacylglycerols, and free fatty acids. Supercritical fluid chromatography/mass spectrometry (SFC/MS) analysis was performed using the Analytical SFC Method Station (Waters) with the Q Exactive mass spectrometer (Thermo Scientific). Full MS/dd-MS 2 (data-dependent MS 2) with the inclusion list, i.e., the parent mass list of the target lipids, was used. The Top 5 method was adopted, i.e., MS 2 was performed for 5 lipid ions in the inclusion list, starting with the ion with the highest intensity. Lipids were identified by the product search mode in Lipid Search (Mitsui Knowledge Industry), which is based on the accurate value of the parent mass and the spectral pattern of MS2.

We investigated the SFC and MS conditions using a standard mixture of lipids. First, we optimized the MS detection conditions such as the sheath gas flow rate, spray voltage, and heater temperature (temperature of the vaporizer). Next, we optimized the MS 2 collision energy. Then, we investigated the separation conditions for lipids such as column type and gradient program. Further, we optimized the identification conditions of Lipid Search. After the analytical method was developed, we analyzed the lipids present in mouse plasma. More than 200 lipid molecular species were detected and identified from each sample.

In conclusion, we demonstrated that the combination of the data-dependent MS 2 scan triggered by the positive or negative parent mass list and Lipid Search software enables the efficient and feasible identification of lipids.

PWe-087
11:10 – 12:20

Simultaneous profiling of polar lipids by supercritical fluid chromatography/tandem mass spectrometry with methylation

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Keywords:
Methylation, Polar lipid, Supercritical fluid chromatography, Triple-quadrupole mass spectrometry

Novel aspects:
Advancement of peak tailing and detection sensitivity of polar lipids by applying TMSD methylation to analytical method based on SFC/MS.

Abstract:
In lipidomics, the simultaneous analysis of diverse lipids with various structures and polarities is too difficult with regard to their separation, detection, and identification. In the previous paper, SFC/MS was applied successfully to analyze 14 types of lipids. However, the analysis of polar lipids was not sufficient due to the peak tailing, low resolution, and low detection sensitivity. Next, trimethylsilyl (TMS) derivatization was applied to advance the peak shape and detection sensitivity of polar lipids. However, several polar lipids such as phosphatidylserine (PS) and lysophosphatidylserine (LPS) were not advanced. Finally, we attempt to use the methylation by TMS-diazomethane (TMSD) and to construct the analytical system for the simultaneous analysis of various and low-abundance polar lipids in a biological sample. Several lipid standards that consist of 6 phospholipids, 6 lysophospholipids, and 7 sphingolipids, dissolved in methanol were applied to simultaneous polar lipid profiling with TMSD methylation. 2 mol/L TMSD in hexane was added to the standard lipids and reacted at 50 °C for 10 min. The reaction was quenched with glacial acetic acid. The methylated lipids were subjected to SFC-QqQ/MS analysis that consists of SFC Method Station and Xevo TQ (Waters, Milford, MA, USA) . In the analysis of biological sample, the TMSD methylation was applied to the lipid extracts with the same method. Simultaneous profiling of 19 polar lipids that consist of phosphatidylcholine (PC) , phosphatidylethanolamine (PE) , phosphatidylserine (PS) , phosphatidylglycerol (PG) , phosphatidylinositol (PI) , phosphatidic acid (PA) , lysophosphatidylcholine (LPC) , lysophosphatidylethanolamine (LPE) , lysophosphatidylserine (LPS) , lysophosphatidylglycerol (LPG) , lysophosphatidylinositol (LPI) ,lysophosphatidic acid (LPA) , sphingomyeline (SM) , ceramide (Cer) , ceramide- 1 -phosphate (Cer 1 P) , sphingosine (So) , sphingosine- 1 -phosphate (So 1 P) , sphinganine (Sa) , and sphinganine- 1 - phosphate (Sa 1 P) was carried out by using SFC/MS with TMSD methyltation. At first, MS/MS method for detecting 16 kinds of methylated polar lipids and 3 kinds of non-methylated polar lipids was optimized by performing the product ion scan to find the precursor ion and fragment ion of each polar lipid. The values of cone voltage and collision energy were also optimized. For the best efficiency of TMSD methylation, the reaction time and temperature were optimized as the reaction at 50 °C for 10 min. By the test of repeatability, RSD % of TMSD methylation was under 8 % . And several columns were investigated for finding the best column for methylated polar lipid profiling. Finally, we successfully construct an effective polar lipid profiling system based on SFC/MS with Inertsil ODS- 4 column by applying TMSD methylation. We also optimized the analysis of non-methylated polar lipids for the comparison between methylated and non-methylated polar lipid profiling. As a result, by applying TMSD methylation, the improvement of peak tailings was resulted in the analysis of 9 polar lipids that consist of PS, PI, PA, LPS,LPI, LPA, C 1 P, So 1 P and Sa 1 P. Furthermore, the LODs of PS, PG, PA, LPS, LPG, LPI, LPA, Cer 1 P, So 1 P, and Sa 1 P were enhanced 7.5-, 5 -, 26.7-, 600-, 6.7-, 116.7-, 500-, 75-, 3000-, and 4500-fold, respectively. Validation study of the developed method represented high reproducibility and high coefficient of correlation. Simultaneous profiling of diverse polar lipids in a biological sample was also performed by this method. This result indicated that the application of TMSD methylation is effective for the analysis of polar lipids by SFC/MS. This developed method is applicable to lipidomics study as a powerful tool, especially targeting polar lipids.

PWe-088
13:30 – 14:40

A screen using DART-MS to identify novel components of a pheromone synthesis pathway in Drosophila melanogaster

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Keywords:
DART-MS, lipids, pheromones, insects, hydrocarbons

Novel aspects:
DART-MS is used for the detection and semi-quantification of pheromones and other lipids from live insects.

Abstract:
Pheromones are molecules produced by an animal that can elicit complex social behaviours (eg, aggression and courtship) from other individuals of the same species. Understanding the biochemical synthesis of pheromones and the role they play in behaviour is useful for elucidating conserved lipid metabolism pathways and may provide novel, species-specific methods for controlling reproduction of pests and disease-bearing insects. The fruit fly, *Drosophila melanogaster*, is a well-studied model organism for which many genetic tools are available. Pheromones of *D.melanogaster* are expressed on the cuticle and have been well-characterized. Analyses of insect cuticular hydrocarbons (CHCs) have conventionally been carried out with gas chromatography coupled with mass spectrometry (GC-MS) . However, sample preparation for GC-MS is time consuming and involves placing whole insects in organic solvent, a process that is lethal to the insects and provides no spatial information. Here, we use a form of ambient mass spectrometry, Direct Analysis in Real Time mass spectrometry (DART-MS) . This method requires little sample preparation and allows rapid analysis of CHCs directly from whole flies. We combined DART-MS with genetic manipulation in order to identify components of the pheromone synthesis and transport pathway in D. melanogaster. Specifically, we are interested in genetic mutations that result in aberrant CHC profiles.

A library of 130 different transgenic fly lines was generated from 65 candidate genes. In each of these lines, the expression of individual lipid metabolism-related genes was genetically knocked down using RNA interference. The pheromone profiles of 5 - 7 individuals from each line were analyzed using DART-MS. CHC profiles of transgenic flies obtained by DART-MS were compared to that of genetic controls to detect changes in CHC profiles. Individual peak intensities were normalized to the most abundant CHC component in each profile. Relative intensity differences were compared across 65 different transgenic lines. Differences that were greater than 2 standard deviations were selected for a secondary screen.

Approximately 10-15 different CHC species could be identified from each individual fly. Profiles between males and females are different. Three positive controls (previously published genes known to affect CHC profile) were identified in the screen. Additionally, in the primary screen, 11 transgenic lines were shown to produce significantly modified CHC profiles for females while 15 transgenic lines showed significant modifications for male CHC profiles. Six out of the 11 transgenic lines identified by the primary screen for females and 10 out of 15 transgenic lines for males were found to contain significant modifications in CHC profiles after a secondary screen with a larger sample size. The type of modifications identified from the secondary screen was consistent with that of the primary screen. In addition, 11 novel genes have been identified that contribute to pheromone synthesis which includes genes involved in the elongation, long chain fatty acid transport, dehydrogenation, oxidation and reduction processes. These results confirm that DART-MS is able to provide accurate and rapid analysis of chemical profiles and is effective as a screening method.

PWe-089
11:10 – 12:20

Inter-strain comparative lipidomics of microalgae using an online multi-dimensional HILIC-RP-UPLC-QToF-MS

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Keywords:
multi-dimensional separation, orthogonal chemistry, UPLC, lipidomics

Novel aspects:
We report for the first time the use of an online high throughput multi-dimensional separation UPLC-QToF-MS system utilizing orthogonal HILIC-RP chemistries for the application of lipidomics and potentially other fields.

Abstract:
Introduction

With the increase of petroleum prices in recent years, alternative forms of biofuels are gaining significant interest as replacements since they are both renewable and cleaner-burning as opposed to fossil fuels. One emerging form of biofuel production is from the cultivation of microalgae. Currently, adoption of commercial scale cultivation has been hindered by factors such as the geography of the land, climate, costs of water replenishment due to evaporative losses, costs of nutrients etc. Many of these factors could be either eliminated or, the associated costs could be minimized by the suitable selection of the microalgal strain from the beginning.

In this study, we compare the lipid profiles, particularly in the glycerolipid class which correlates to the potential biofuel yield, of various strains of a chlorophyte using a novel orthogonal two-dimensional HILIC-RP UPLC coupled to quadrupole time of flight mass spectrometry.

Experimental

Lipids were extracted from the harvested algal biomass using the Folch method. The extracted sample was injected into a 2 D-UPLC system with a HILIC column as the first dimension and a charged surface C_{18} column as the second dimension of separation. An short isocratic run using acetonitrile and water buffered with ammonium acetate was utilized for the HILIC method. A trapping column was placed in line to trap the lipids of interest eluting off the HILIC column. The trapped lipids were then back flushed into a charged surface C_{18} column running a gradient of acetonitrile, water and isopropanol buffered with ammonium acetate. Accurate mass data from both precursor and fragment ions of the lipids were collected simultaneously by a Q-ToF MS running in MS^e mode. Lipids were identified using SimLipid and comparative statistical analysis between the various strains was performed using MarkerLynx™.

Preliminary Results and Discussion

Traditional direct infusion and reversed phase LC-MS methods suffer from poor sensitivity of low abundance but biologically important lipids due to the co-elution of interferences which could include lipids from other classes i.e. highly abundant glycerophospholipids. Recently developed HILIC-UPLC methods show improved class separation but again, the individual lipids within each class co-elute and thus interferences also form.

We see improved detection and identification of the glycerolipids as the starting high organic mobile phase composition flowing through the HILIC column allowed only the hydrophobic classes to elute off the column while retaining the more polar phospholipids. These classes of lipids were then further separated on the charged surface C_{18} column according to their hydrophobicity or alkyl chain length void of any other interferences. The orthogonal two dimensional approach utilized here effectively doubled the peak capacity of the separation method. This improved both the resolution and dynamic range and thus the confidence level of lipid identification was higher when the data was analysed by SimLipid.

As more lipids were identified per sample due to the improved sensitivity of the method, there was a greater differentiation between the various strains than previously reported from data obtained by direct infusion ion-trap MS.

Conclusion

The novel 2D-UPLC-QToF-MS method described here eases the handling of complexity and dynamic range issues seen in traditional lipid analysis methods, combining the benefits of orthogonal chemistries. Data obtained from the application of this method in algal lipidomics would be critical for future strain selection in commercial scale cultivation, potentially saving companies resources that would have been wasted from the adoption of incorrect or low-yielding strains.

PWe-090
13:30 – 14:40

Lipid identification through MS and MS/MS analyses combined with new database search software

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Keywords:
Lipid identification, Lipid analysis software using MS and MS/MS data, MALDI-TOF/TOF and -FTICR, ESI-QTOF and ion trap

Novel aspects:
An automated lipid identification platform was validated with various mass spectrometry platforms.

Abstract:

Introduction:
Lipids are a large and diverse group of molecules that include fats, sterols, phospholipids, glycerides and many others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules. These findings have led to an increasing interest into lipid analysis at large scale during the past years, also coined "Lipidomics". The huge structural variety makes their analysis quite demanding. Today mass spectrometry and liquid chromatography mass spectrometry represent versatile tools in the field of lipid analysis, also offering the possibility for molecular structural identification. One of the most important aspects of mass spectrometric lipid data analysis is the structural identification of lipids using precursor and MS/MS data.

Methods:
Glycerophospholipids standards (Avanti Polar Lipids) from different lipid classes were analyzed, such as phosphatidic acid (PA 16:0-16:0), glycerophosphocholines (PC 18:0, PC 16:0-18:1 (9Z), PC 18:1-18:1 (6Z), PC 18:1-18:1 (9Z)), glycerophosphoethanolamines (PE 16:0-18:1 (9Z)), glycerophosphoserines (PS 14:0-14:0), glycerophosphoglycerols (PG 16:0-16:0) and sphingomyelins (SM 16:0). For each lipid mass spectra in positive and negative mode were acquired on FTICR, ion trap, Q-TOF and MALDI-TOF/TOF instruments and raw data exported to the SimLipid software (Premier Biosoft). Individual lipids were identified by a structure database search using the SimLipid software.

Results:
In this study we analyzed a number of standard lipids using MALDI-TOF/TOF, MALDI-FTICR and ESI-QTOF and ESI-iontrap (infusion) techniques. For most analyses positive ionization mode was used, negative mode was successfully applied to the analysis of lipids with an acidic characteristics such as PA, PG and PE.
The lipid identification software used MS and MS/MS spectra containing peak list data for lipid profiling and structural elucidation. The program accepts experimental MS and MS/MS data obtained by mass spectrometry in text formats (e. g. MS Excel, mzData and mzXML) and native file formats from all used mass spectrometers. Lipids were identified by searching precursor molecular weights and MS/MS fragments against known lipid structures available in a database provided by SimLipid and fragment masses calculated based on the lipid structures.
All lipids were unequivocally identified by database searching (typically rank 1 to 3 in the result list) independent of the mass spectrometer used and the ionization technique involved. The lipids were identified within MS/MS error tolerance settings that are adequate for the various mass analyzers (0.005-0.2 Da). The various tested lipid classes and their covalent gross structures, such as the head group type and the fatty acyl chain composition were correctly attributed in the search results in all cases. However, the localization of double bonds by this approach was not possible due to lack of specific fragments; here chemical derivatization remains to be required.
An instrument independent platform for lipid identification was established in this work, which can now be used in lipid characterization or lipidomics by LC-MS/MS, infusion-ESI or TLC-MALDI (thin layer chromatography) workflows.

PWe-091
11:10 – 12:20

Lipidomic profiling of mammalian brain and biofluids by RP-LC/MS: Global approaches and structural analysis using LTQ-Orbitrap.

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CEA, Gif-sur-Yvette, France

Keywords:
Lipidomic, tissue, biofluids, LC-MS

Novel aspects:
We discribe an efficient methodology to profile lipid molecular species in biological matrices

Abstract:
Lipids are essential cellular constituents of all mammalian cells that have many critical roles in cellular functions. If many families of lipids such as glycerophospholipids, sphingolipids, glycerolipids and sterols are mainly present in outer layer of cell plasma membranes, other lipids are also involved in energy storage or participate to cell signaling. Lipid metabolic or immunological alterations may have serious pathological consequences such as neurometabolic or neurodegenerative diseases. Due to the high structural diversity of lipid families including polar or neutral molecules, a complete molecular profiling of biological matrices stay a challenge in lipidomic for qualitative and quantitative method developments. Complex mixture analysis of lipids using mass spectrometry technologies was reported since 1990s and actually recent developments in electrospray ionization mass spectrometry (ESI/MS) using high resolution system instrumentation have rapidly expending the research on this field. Lipidomic analysis can be performed using "shotgun " approaches without any previous chromatographic separation, or using LC/MS to analyze a larger number of molecules.
A comprehensive lipidomic methodology based on lipid fractionation using SPE cartridges (LC-NH 2) coupled or not with RP-LC/MS was implemented to build a lipid database including exact mass, retention time and MSn spectra for further development of a robust lipid profiling method. Due to the high heterogeneity of lipid species in biological matrices, brain tissues, human plasmas and cerebrospinal fluids (CSF) were selected in this study.
Brain lipid extracts were obtained using a Folch modified partition [1] from adult Sprague-Dawley rat and human biofluids were obtained using modified Bligh and Dyer extraction [2] . The upper phases containing ganglioside species were desalted using C18 columns and pooled with the lower phases which contain the other lipid families. An aliquot of total lipid extract (TLE) was redissolved in chloroform and a lipid fractionation was performed using LC-NH 2 columns to separate crudely lipid classes [3] . Six fractions were obtained and compared with TLE using a RP-LC/MS. TLE and fractions were separated, during 32 min per run, on an Ascentis Express C18 2.1x150mm 2.7µm column connected to an Accela quaternary HPLC pump coupled with an LTQ-Orbitrap. The tune methods were optimized using lipid standards.
All fractions obtained from LC-NH 2 columns were analyzed in postive and negative ion modes by direct infusion ESI-MS and LC/MS and compared with the total lipid extract of all biological matrices. LC-NH 2 fractions were used to simplify lipid species identification in order to build a homemade lipid database. In a single LC/MS run, numerous lipid species were identified in both fractions and TLE. In presence of ammonium formate, in positive ion mode, salt adducts are drastically reduced (Na, K) and many lipid classes such as phosphatidylcholines and their lyso-forms, ceramides, cerebrosides and sphingomyelins were detected as [M+H] + ions. Other lipid species (mono- di- and tri-glycerides, and sterols) are also detected as [M+NH 4] +. However, to limit the presence of phospholipids and neutral lipids in blank samples before and after sample analysis, a wash with organic solvents must be used during the run. In the negative mode, numerous classes of lipids were also detected such as free fatty acids, phosphatidylethanolamines and lyso-forms, sufatides, phosphatidylinositols, phosphatidylserines, phosphatidylglycerols, and gangliosides as [M-H] - ions. In this study, numerous lipid species was identified using high resolution mass spectrometry. As an example, 250 phospholipid isoforms, 150 sphingolipid isoforms and 250 neutral lipid isoforms are listed in the database. Interestingly, many lipid isoforms was found in only one investigated biological matrix.

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PWe-092
13:30 – 14:40

Development of ultra-high sensitive detection method of sphingosine-1-phosphate and its related sphingoids by LC/MS/MS

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Keywords:
Sphingoids, Sphingoid-1-phosphates, Laser micro dissection, Brain, LC/MS/MS

Novel aspects:
We have developed a method for analyzing intracellular kinetics and disposition of sphingoids and sphingoid-1-phosphates at a cellular level, combining a LMD with an ultra-high sensitive method using LC/MS/MS.

Abstract:
Introduction
Sphingoids and sphingoid-1-phosphates are endogenous compounds, and their function of lipid mediator has been noticed in these days. However, the biological kinetics and intracellular disposition at a cellular level have not been demonstrated. A micro dissection is useful tool for analyzing the distribution of intercellular endogenous compounds with cytological staining. However, it is difficult for determining sphingoid-1-phosphates because phosphate groups are easily removed and unstable in biological samples. Therefore, it has been required ultra-high sensitive detection and stable sample preparation method to determine sphingoids and sphingoid-1-phosphates with a micro dissection sample.
Purpose
In this study, we have developed a method for analyzing biological kinetics and intracellular disposition of sphingosine-1-phosphate and its related sphingoids at a micro level, combining a laser micro dissection (LMD) system with an ultra-high sensitive detection method using LC/MS/MS.
Methods
An ultra-high sensitive determination method of sphingosine-1-phosphate (S1P) and 5 sphingoids has been developed by LC/MS/MS. LC separation was optimized using a NANOSPACE SI-2 HPLC system (Shiseido). MS/MS was conducted on a TSQ Quantum Ultra (Thermo Fisher Scientific) triple quadrupole mass spectrometer equipped with a heated ESI source. The detection was selected ion monitoring mode. The symmetry factor, theoretical plate number and signal to noise (S/N) were evaluated to establish an ultra-high sensitive method. To indicate the effect of the presented method, brain samples were prepared from male C57BL6 mouse and dissected the brain section by LMD system (LMD 6000, Lica). After perfusion with PBS, brain was quickly removed and buried into optimal cutting temperature compound. A 10 µm slice of mouse brain has been prepared by cryostat (Microm HM550, Thermo fisher scientific), and put on a slide glass. Then slice sample was dissected several brain regions by LMD system. The dissected samples were prepared by simple methanol deproteinization.
Results and discussion
We have improved the sample preparation method using on-line column switching, and particularly focused on changing the HPLC flow passages. The optimized online column-switching system consisted of a trap column, a CAPCELL PAK ACR (1.0 mm i.d. × 35 mm, 3 µm, Shiseido) and an analytical column, a CAPCELL PAK ACR (1.0 mm i.d. × 150 mm, 3 µm). The elution mobile phase were 5 mmol/L ammonium formate ($HCOONH_4$)-H_2O (pH 4) (A), 5 mmol/L $HCOONH_4$-H_2O/acetonitrile (5/95, v/v; pH 4) (B), and loading was A/B (70/30, v/v), and the flow rate was 60 µL/min. These conditions had the best performance of symmetry factor (0.99), theoretical plate number (213912) and S/N (14581) of 100 nmol/L std. solution, and no peak tailing was observed on the chromatograms. A sensitive and selective assay was developed with a 50 pmol/L lower limit of detection for S1P and its related sphingoids. All of dissected samples of mouse brain were detected S1P and some of sphingoids, and samples were required 5,000 - 20,000 cells to detect by LC/MS/MS. The concentration of S1P in cerebellum, cortex and nigra were 1.22±0.03, 0.40±0.13 and 1.53±0.16 nmol/L, respectively.
Conclusion
We have developed an ultra-high sensitive method for S1P and its related sphingoids. The dissected brain samples were stable for S1P on LMD system. The distribution of S1P in the brain regions was demonstrated. These results are indicated that the present method is powerful tool for analyzing the intracellular distribution of S1P and its related sphingoids in tissue dissection samples.

PWe-093
11:10 – 12:20

Mass spectrometry based assessment chimeric mouse liver metabolite profiles following oral dosing of troglitazone

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Keywords:
LC/MS, metabolomics, accurate mass, profiling, MetID

Novel aspects:
Lipid metabolite profiling used to characterise animal groups as a tool to gauge sample integrity for drug metabolism experiments.

Abstract:
The use of chimeric (PXB) mice, with up to 85% humanized livers, have become well established in the study of drug metabolism and disposition in which the majority of the hepatocyte population of the mouse liver has been replaced by human hepatocytes. Expression of human Phase I and II metabolic enzymes and hepatic transporter proteins enable identification of drug metabolites that may not be detected by other means. Histological based methods to assess the percentage human tissue can offer a visual gauge of the amount of human tissue. For known compounds with metabolism characteristic to human, drug metabolites can also offer a guide as percentage humanised tissue in chimeric livers. Endogenous lipid differences, also occurring between PXB and wild type (SCID) mice may also offer a guide as percentage humanised.
To assess the viability of the chimeric PXB mouse troglitazone (TGZ) was dosed orally over 7 days. In pre-clinical studies TGZ showed inter-species differences in metabolism particularly in sulfation and glucuronidation pathways. The present study evaluated the metabolic profile of troglitazone and endogenous metabolites in the PXB compared to SCID (severe combined immunodeficiency) mouse model by high mass accuracy MS/MS analysis.
Liver extracts from SCID and PXB mice were analysed using a high resolution LC/MSn system (Nexera LC coupled with a LCMS-IT-TOF ; Shimadzu Corporation) . Aqueous extracts were analysed using a Phenomenex Kinetex XB C18 1.7um (2.1x100mm) held at 30C ; components were separated with a 40 minute gradient (mobile phase was A - 0.1% formic acid solution + 10mM ammonium acetate and B - acetonitrile + 0.1% formic acid) at a flow rate of 0.6mL/min. The LCMS-IT-TOF acquired negative MS and MS 2 data (m/z 150-800) .
In preclinical studies, TGZ human hepatic metabolism is broadly similar to rat, dog, marmoset and cynomolgus monkey with the sulfate conjugate being the most abundant metabolite detected while glucuronidation was predominant in mouse. Consistent to published results, in this study the sulphate metabolite was measured at higher levels in humanised whereas the glucuroniode was reduced. Marked differences in endogenous metabolic profiles were also observed between the humanized and wild type mice with several glycerophosphocholine compounds both elevated and decreased up to twenty fold difference in peak area. In addition, several fatty acid compounds were decreased only in troglitazone treated animals. These results may in future studies offer the possibility to provide a non-histological based estimation of percentage humanised liver whilst also providing an indication of endogenous metabolite changes occurring from drug administration.

PWe-094

13:30 – 14:40

High-throughput lipid profiling system for dried plasma spots by using online-supercritical fluid extraction-supercritical fluid chromatography/ mass spectrometry

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Keywords:

supercritical fluid extraction, supercritical fluid chromatography, Dried plasma spot, lipidomics, phospholipid

Novel aspects:

Phospholipids in dried plasma spot could be analyzed without any pretreatment in 15 min using online-SFE-SFC/MS/MS. 172 phospholipids from only 3 µl plasma could be detected.

Abstract:

Although biological analysis is usually performed using plasma obtained by venipuncture, the use of dried blood spots (DBS) has grown in popularity in the clinical and pharmaceutical communities over the past decade as an alternative sampling procedure. DBS and dried plasma spots (DPS) analysis is available as a diagnostic tool for genetic diseases and is useful for the screening of biomarkers.

In this study, a high-throughput analytical system based on online-supercritical fluid extraction-supercritical fluid chromatography with tandem mass spectrometry (online-SFE-SFC/MS/MS) was developed for phospholipids profiling of DPS. This system is able to simultaneously perform extraction and separation, allowing phospholipids that have common polar head groups to be analyzed. We investigated conditions of the online-SFE-SFC/MS/MS. First we optimized SFC and MS conditions to separate and detect the phospholipids such as column type and collision energy. Next we optimized online-SFE-SFC conditions using mouse plasma to extract and separate these phospholipids such as modifier concentration and extraction time.

In the result phospholipids in only 3 µl of plasma can be extracted in 5 min and analyzed within 15 min using this system. A total of 172 phospholipids, including phosphatidylcholine (PC) , lysoPC, sphingomyelin (SM) , phosphatidylethanolamine (PE) and lysoPE, were annotated, and 78 phospholipids were analyzed with good repeatability. The online-SFE-SFC/MS/MS, which is able to perform high-throughput lipid profiling analysis for clinical diagnosis and drug discovery, may also be suitable for the screening of biomarkers.

PWe-095
11:10 – 12:20

Systems based LC-MS metabolite profiling of mice treated with ethanol enriched liquid diets

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Keywords:
LC/MS, metabolomics, profiling, alcohol, ethyl glucuronoide

Novel aspects:
Using a systems based approach to metabolite profiling, significant alcohol marker compounds were detected by accurate mass MetID software.

Abstract:
Through measurement of liver, plasma and urine, a systems based approach to metabolite profiling can provide a perspective of change in endogenous compounds through modified dietary factors. Previous studies into the harmful effects of alcohol have utilised the intragastric feeding models in which animals have surgically inserted feeding cannulas. In this study a less invasive four week ethanol enriched liquid diet was evaluated as an alternative vehicle to modified diet. To gauge the impact of ethanol exposure, known ethanol metabolites were monitored by high resolution mass spectrometry to monitor metabolic change.
Animal liquid diets (Dyets, Inc., Bethlehem, PA) were administered to control mice (Dyets #710079 and ethanol treated mice (Dyet #710301) . The percentage alcohol derived calorie in the alcohol diet was 10% for week 1, 20% - week 2, 35% - week 3 and 4. When 35% of the calorie was derived from alcohol, alcohol concentration was ~6.5% (v/v) . Urine, plasma and liver tissue samples were separated by Nexera UHPLC (Shimadzu) using a Phenomenex Kinetex column (XB C18 1.7um 2.1x100mm) over 12 minutes (A - 0.1% formic acid solution + 20mM ammonium formate and B methanol + 0.1% formic acid + 20mM ammonium formate) at a flow rate of 0.7mL/min. LCMS-IT-TOF was used to acquire high mass accuracy MSn data.
In this current study ethylated lipid marker compounds were detected, consistent to previous work published from intragastric feeding models. The profound lipid differences observed from previous work were markedly reduced in the current liquid diet study with the emergence of ethylated lipid markers at week 4 but not at week 2. These included ethyl arachidonate, ethyl linolate and ethyl DHA which were not detected in control or 2 week samples. In broad agreement to previously published work, retinol palmitate and retinol were also significantly reduced in ethanol treated animals. Comparing urine, plasma and liver tissue, elevated levels of two phosphatidylcholine were measured at significantly elevated levels in urine and liver and to a lesser extent in plasma also in 4 week ethanol treated animals. Urine samples were found to contain ethanol metabolites including ethyl sulphate and ethyl glucuronide, metabolites commonly used to determine recent ethanol consumption. The implications of this targeted metabolomics study suggest that the level of alcohol consumption in this work was lower than in the previous experiments. It may also provide marker compounds that highlight prolonged low level continuous alcohol consumption and possible indicators of alcohol addiction in humans.

PWe-096
13:30 – 14:40

Identification of Insect Species by Direct UV-Laser Desorption/ Ionization Mass Spectrometry of Cuticular Lipids and Pheromones

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Keywords:
LDI, pheromone, lipid, hydrocarbon, identification

Novel aspects:
Direct UV-LDI MS of cuticular lipids from related species of insects reveals distinct chemical profiles that could be used for species identification and evolutionary mapping

Abstract:
Introduction
Insect cuticles are covered with a lipid layer consisting of hydrocarbons, fatty acids, and triglycerides. Many of these compounds serve as pheromones, signaling molecules that influence social behaviors such as mate choice and aggression. Because of their unique behavioral properties, many pheromones are species-specific and sex-specific. As such, pheromone lipid markers can be used for biotyping of insects and could be particularly helpful for identifying cryptic species. The standard method for cuticular hydrocarbon analysis is gas chromatography MS. We recently showed that UV-laser desorption/ ionization (LDI) MS could be used as a complementary analytical method (1). UV-LDI MS detects larger, polar compounds and allows spatially-resolved profiling of single insects. We show here that UV-LDI MS can be used for rapid biotyping of different drosophilid (fruit fly) species based on cuticular lipid profiles.

Methods
Intact, individual adult virgin males and females from 14 different drosophilid species and populations were analyzed. The UV-LDI MS measurements were performed using a modified ion source of an orthogonal TOF mass spectrometer equipped with a N_2 laser. 6-8 flies from each species and of each sex were mounted on a custom-built sample plate and analyzed under elevated source pressure conditions (2-4 mbar). No matrix was applied. Profiles of individual body parts were obtained with a spatial resolution of ~200um.

Preliminary data
The lipid profiles of both closely- and distantly-related *Drosophila* species and populations show clear quantitative and qualitative differences. Among the different kinds of lipids identified from the cuticular surface are C_{20}-C_{37} length unsaturated hydrocarbons (including alkenes, dienes, trienes), oxygen-containing hydrocarbons, and triglycerides. Closely-related species and populations show quantitative rather than qualitative differences. In contrast, distantly-related species exhibited striking differences in lipid profiles, particularly in the pheromone components of male sex glands. Males and females of all species tested exhibited distinct sexually dimorphic lipid compositions. The cuticular profiles of recently-mated females also showed changes, indicating that some male-specific compounds were transferred from males to females during copulation. These results indicate that cuticular lipids of insects can be used as biomarkers specifying species, sex, and mating status. Such information is important for species identification and may be used to trace evolutionary relationships.

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PWe-097
11:10 – 12:20

Systems-Based Approaches to Elucidating Gender-Specific Mechanisms in the Etiology of Atherosclerosis

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Keywords:
eicosanoid, lipidomics, metabolomics, atherosclerosis, cardiovascular disease

Novel aspects:
A systems-based approach integrated transcriptomics, lipidomics, metabolomics and lipid mediator data from carotid plaques. Multivariate-statistical analyses highlighted lipid mediator pathways that evidenced gender-specific shifts at the gene and lipid level.

Abstract:
Cardiovascular disease is the major cause of premature death in Europe, resulting in > 4 million deaths annually. Cardiovascular disease is often regarded as a "men's " disease ; however, it is the leading cause of mortality among women. In particular, since 1984, more US women than men have died annually from cardiovascular disease. Studies have suggested a key role for oxidized fatty acids (oxylipins) in inflammatory reactions of atherosclerosis ; however, it is still unclear to what extent pro- and anti-inflammatory factors determine whether an atherosclerotic lesion develops into a stable plaque orruptures, leading to stroke or myocardial infarction. To further probe the etiology of plaque development and subsequent rupture, we performed a combination of lipidomics and metabolomics as well as targeted oxylipin, freefatty acid and endocannabinoid lipid mediator profiling in human carotidatherosclerotic plaques and matching circulating plasma. These data were combined with sonographic gray-scale median plaque imaging and AffymetrixGeneChip® data as well as patient clinical parameters to develop a multivariate model of plaque gender-specificity. PCA analysis showed that plaque and plasma have unique composition (R^2 =0.54, Q^2 =0.41) , which was mainly driven by oxylipin levels, despite the fact that endocannabinoids represent >90 % of the lipids measured. Orthogonal projections to latent structures (OPLS) analysis based on gender resulted in a robust oxylipin-driven model with high predictive power, which was specific for plaque (R^2 =0.87, Q^2 =0.43) . No model could be generated using data from circulating plasma. The most important variables driving the separation between genders were primarily products of the12/15 lipoxy genase pathway (12/15-LOX) , suggesting gender-specific differences in this key pathway. These trends were supported by gene set enrichment analyses (GSEA) showing enrichment in linoleic- and eicosanoid-specific pathways in plaque, but not plasma. Accordingly, the observed changes in lipid and gene data agreed in the vector and magnitude of the alterations, suggesting that gender-specific pathways do exist in carotid plaques. Imaging data suggested that plaques in women had distinct morphological differences, with women having overall more calcified plaques. Collectively, results point to gender-specific shifts in inflammatory lipid species as well as morphological differences that could potentially explain the higher incidence of cardiovascular disease in women.

PWe-098
13:30 – 14:40

Molecular lipidomic workflow solution identifies a single lipid marker for the diagnosis of statin myalgia

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Keywords:
Molecular lipidomics, statin, myalgia, high-throughput, biomarker

Novel aspects:
Molecular lipidomics identifies diagnostic lipid markers

Abstract:
Statins, globally most prescribed drugs, can cause a wide range of muscular side effects, from non-specific myalgias to the full-blown rhabdomyolysis. Statin intolerance may substantially reduce treatment compliance and statin efficacy in preventing cardiovascular end points. The prevalence of myotoxicity is clearly higher in clinical practice than has been shown in clinical trials. Statin myotoxicity is underdiagnosed partially because the currently used biomarkers such as creatine kinase (CK) are not sensitive enough to detect most patients who develop muscle weakness or pain. Here, we assessed proprietary state-of-the art molecular lipidomics high-throughput workflows for the discovery of more sensitive biomarkers for statin induced muscle toxicity.
Plasma samples were obtained from statin users with or without muscle complaints (n=334) . The main muscle symptoms were myalgia and weakness. The lipidomic assessment was carried out using an in-house Zora lipidomics solution composed of multiple tailor-made lipidomics platforms supported by Vostok, bioinformatic processing tools, providing stringently quality controlled outputs of molecular lipids in absolute amounts. Plasma samples were carefully prepared using a lipidomics customized robotic sample preparation and extraction workflow, followed by high throughput quantitative shotgun and targeted lipidomic workflows assessed on QTRAP 5500 technology equipped with a robotic nanoflow ion source and UHPLC systems, respectively.
The molecular lipidomic assessment revealed that the concentration of 12-hydroxyeicosatetraenoic acid (12-HETE) was significantly higher in statin intolerant patients compared to patients with no clinical signs ofmuscular side effects (median difference +125%, $p = 1.0x10^{-13}$) . 12-HETE discriminated cases and controls equally well both in men and women. Taken together, the proprietary in-house molecular lipidomics solution identified 12-HETE as a sensitive and specific biomarker for statin-induced myalgia. This single lipid marker may serve as a new tool for physicians, and will help them to rationally select treatments for patients sensitive to statin induced adverse muscle side-effects. This study successfully demonstrates the value of stringently executed quantitative molecular lipidomics in biomarker discovery.

PWe-099
11:10 – 12:20

Development of comprehensive glycerophospholipid profiling methods using liquid chromatography / high-speed triple quadrupole mass spectrometry

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Keywords:
Lipidomics, glycerophospholipids, LC/MS, SRM

Novel aspects:
High-speed triple quadrupole mass spectrometry enabled high-throughput and comprehensive profiling of glycerophospholipids.

Abstract:
Glycerophospholipids are important as structural and functional components of cellular membranes. Based on the polar headgroup at the *sn*-3 position of the glycerol backbone, glycerophospholipids are divided into distinct classes such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), and cardiolipin. Each glycerophospholipid class consists of numerous molecular species due to a variety of the linkage between the fatty acid (FA) chains and the *sn*-1 position of the glycerol backbone (i.e. acyl-, alkyl-, and alkenyl-linked fatty chains), and the complexity of the combinations of fatty acids at the *sn*-1 and *sn*-2 positions which may differ in carbon number and degree of unsaturation. The major fatty acids in glycerophospholipids can vary in length from 14 to 22 carbons and may have 0 to 6 double bonds. In theory, over a thousand different glycerophospholipids molecular species are possible. Compositions of glycerophospholipids differ among tissues as well as cell types. Such diversity may be critical for various cellular functions, although the roles of the various molecular species of each class of glycerophospholipids are still unclear.

Recent advances in mass spectrometry have provided the means for rapid and sensitive analysis of cellular lipids. We developed methods to perform lipid profiling utilizing the enhanced capabilities of a recently developed "high-speed " triple quadrupole mass spectrometer with high-speed scanning, fast polarity switching, and short dwell time. Two liquid chromatography / electrospray ionization/mass spectrometry (LC/ESI/MS) methods were compared for measuring cellular glycerophospholipids. One was a multiple scanning method in which several precursor ion scans and neutral loss scans in both positive and negative ion modes were performed in one analytical run.. The other method was based on SRM (selected reaction monitoring) in which hundreds of glycerophospholipid species are measured simultaneously.

Precursor ion scans and neutral loss scans are commonly used to identify specific polar head groups of glycerophospholipids. SRM-based approaches are routinely used to analyze many small molecules such as drug metabolites, hormones and pesticides with high sensitivity and throughput and are also gaining popularity in proteomics because of the higher sensitivity than scanning methodologies. Here, we compare sensitivities and coverages of glycerophospholipids detected using both scan-based and SRM-based methodologies with various scan speeds and dwell times and discuss advantages and disadvantages of each approach.

PWe-100
13:30 – 14:40

Development of a comprehensive detection method for quantifying eicosanoids using high-speed liquid chromatography / mass spectrometry

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Keywords:
Lipidomics, eicosanoid, LC/MS, SRM

Novel aspects:
A comprehensive and high sensitive detection method of eicosanoids, their metabolites and isomers using a novel LC/MS system was developed.

Abstract:
Eicosanoids are generated from arachidonic acid (AA) as lipid mediator in various pathological and physiological events of mammalian organisms. Enzymatic oxidation of AA by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 including non-enzymatic oxidation induce synthesis of various eicosanoids such as prostaglandins (PGs), leukotrienes (LTs) or hydroxyeicosatetraenoic acids (HETEs). Isoprostanes (iPs) known as oxidative stress biomarkers can be generated via chemical reaction of reactive oxygen or nitrogen species. Comprehensive analysis of eicosanoids and their metabolites or isomers is expected to serve as a key technology for disease studies. Selected reaction monitoring (SRM) using liquid chromatography/mass spectrometry (LC/MS) has been widely used for detecting eicosanoids[(1-3)]. Although SRM is a selective and sensitive detection method, performing a large number of SRM channels can often cause poor sensitivity. To analyze eicosanoids comprehensively without compromising sensitivity, we developed a new quantitative method using a high-speed LC/MS system.
The analytical system consisted of *Nexera* UHPLC system and triple quadrupole (TQ) mass spectrometer LCMS 8040 (Shimadzu corporation). The TQ-MS has a new type of collision cell (UFsweeper™) which emits product ions without decreasing their velocity that enable to detect product ions with keeping sensitivity even if a few milliseconds (ms) were set for one SRM transition such as 1 ms dwell time and 1 ms pause time. Over 50 eicosanoids and their metabolites including not only PGs, LTs, HETEs or iPs such as 8-iso PGE_2, but also platelet activating factor PAF were purchased from Cayman Chemical. Samples were dissolved in methanol. We evaluated several HPLC columns such as Shim-Pack ODS XR-III (2.0 × 150 mm, Shimadzu Co.), CAPCELL PAK ODS IF-II (2.1 × 100 mm, Shiseido Co.) or Kinetex C8 (2.1 × 150 mm, Phenomenex). A typical flow rate using Kinetex column and gradient of B concentration were 0.4 mL/min and 15% (0 min) 35% (10 min) 85% (20 min), respectively.
A mixture of 50 eicosanoids was separated and detected using the LC/MS system after SRM optimization for each compound. All compounds resolved in 20 min. Most of compounds detected in negative mode. Several lactone or ethanolamide species were appeared in positive mode. Enantiomers such as PGF_{2a} and *ent*-PGF_{2a} were not resolved. Both LTC_4 and PAF peaks were broadened when Shim-Pack ODS XR-III column was used. The broadenings were improved using CAPCELL ODS PAK IF-II column. Further improvement of peak shape was observed using Kinetex C8 column. Therefore we used Kinetex column for following quantitative analysis. Setting 10 ms for each transition to over 50 SRMs including twice of polarity switching (15 ms × 2), linear calibration range spanning from 0.5 to 1000 pg was obtained (R^2> 0.999). Limit of quantifications (LOQ) were below 1 pg for a number of eicosanoids and isomers including 8-iso PGE_2, PGD_2, LTB_4 and several HETEs. To examine the sensitivity under ultimate short transition, we set 2 ms transition including 1 ms dwell and 1 ms pause time to 100 SRM transitions. Consequently, LOQ of PGE_2 was 1 pg even when such a high-speed SRM set was applied. These results suggest that several hundreds of eicosanoids can be simultaneously detected with up to 1 pg sensitivity. We will extend the method to cover a large number of fatty acid metabolites and other mediators without compromising throughput and sensitivity.

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PWe-101

11:10 – 12:20

Formation and collision-induced dissociation (CID) of unsaturated fatty acid-alkali metal anionic complexes: A new approach for determining double bond position

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Keywords:

Fatty acid analysis, collision-induced dissociation, double bond position

Novel aspects:

Presented for the first time is the collision-induced dissociation of fatty acid-alkali metal anionic complexes and two methods for producing these anionic complexes.

Abstract:

Fatty acids are arguably the most important lipid class and are the building blocks for the majority of lipid classes. Isomeric fatty acids differing only in double bond position are common in nature and include ; 18 : 1 *n*-9 /*n*-7, 18 : 3 *n*-3 /*n*-6, 20 : 3 *n*-3 /*n*-6 / *n*-9 and 22 : 5 *n*-3 /*n*-6 (where '*n*' defines the location of the terminal double bond from the methyl end) . Fatty acids are essentially long-chain carboxylic acids and therefore readily produce [M-H]- ions upon electrospray ionisation (ESI) . The collision-induced dissociation (CID) of fatty acid [M-H]- ions results mainly in the neutral losses of water and carbon dioxide while isomer-specific product ions are not observed using ion-trap mass spectrometry. This precludes the "*de novo* " determination of double bond position. Consequently, the CID of *cationic* fatty acid-metal ion complexes has been extensively studied and has, in general, proved more successful in providing rich structural information.

We present here, for the first time, the CID of *anionic* fatty acid-alkali metal complexes. We have found that fatty acid [M-2H+Alk]- anions (where Alk = Li^+, Na^+, K^+) may be generated efficiently by two distinct methods, these are ; 1) Electrospray ionisation of a solution containing a fatty acid and alkali metal fluoride to form the [M-H+AlkF]- anion. CID of the [M-H+AlkF]- anion results in the formation of the [M-2H+Alk]- anion via the neutral loss of HF. Or 2) direct formation of the [M-2H+Alk]- anion by electrospray ionisation of a solution containing a fatty acid and alkali metal hydroxide, bicarbonate or carbonate. The CID of [M-2H+Alk]- anions (in either an MS^3 or MS^2 experiment) results in extensive fragmentation of the fatty acid acyl chain providing a series of ions which allow the assignment of double bond position (s) .

Typically, fatty acids are deprotonated at the carboxylic acid functional group during, or prior to, negative ion electrospray ionisation to form [M-H]- anions. The formation of [M-2H+Alk]- anions requires the removal of an extra proton which can only occur from the fatty acid acyl chain. We propose that this deprotonation occurs at the bis-allylic position (s) of polyunsaturated fatty acids (or the allylic position of monounsaturated fatty acids) resulting in the formation of a resonance-stabilised carbanion. Strong interactions between the alkali metal cation and resonance-stabilised carbanion likely prohibits the migration of the charge site and resulting randomisation of double bond position (s) . Upon CID, charge-driven fragmentations at the site of the resonance-stabilised carbanion then provides product ions indicative of the original double bond position (s) .

These results essentially lay the foundation to previously inaccessible negative ion chemistry. Furthermore, the negative ion approach to determining double bond position by low-energy CID presented here may be thought of as complementary to the CID of dilithiated fatty acid cations ; an established positive ion-ESI method for determining double bond position

PWe-102
13:30 – 14:40

Phosphoinositides analysis by liquid chromatography and mass spectrometry

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Keywords:
Phosphoinositides, lipidomics, mass spectrometry

Novel aspects:
The analysis of phosphoinositides is still a challenging field in the lipidomics world. In this work we developed new methods to make this kind of measurements faster and more sensitive.

Abstract:
Phosphoinositides are important lipid mediators of signalling events in all cellular compartments. They play a key role in the attachment of the cytoskeleton to the plasma membrane, exocytosis, endocytosis, membrane trafficking and enzymes activation. The analysis and quantification of biologically important lipids became a routine process in lipidomics, however the phosphoinositides analysis has not progressed at the same rate and it is still a challenging research field. This is due to their low cellular concentration, their highly charged head groups that affects extraction and detection and their presence as a complex with phosphoinositide binding proteins. In this work, new approaches to examine native and derivatized phosphoinositides in biological samples, by HPLC and nanoLC-ESI-MSMS, are presented.

PWe-103

11:10 – 12:20

Secondary metabolite profile of Fusarium oxysporum by LCMS using QTOF,different sources, ion modes and metal solution post-column addition

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Keywords:
Fungal metabolite profile ; metal complexation

Novel aspects:
The post-column addition of metal solutions, together with the use of different sources and ion modes for metabolic profiling, enabled the differentiation of isomers such as cyclosporine and isocyclosporine A

Abstract:
Metabolite profiling is crucial for many aspects of fundamental research and industrial applications of filamentous fungi and other microorganisms. The use of a quadrupole - time-of-flight mass spectrometer has proved to be very suitable for this purpose due to its mass high resolution capability, and ESI is the most widely used ionization method in both positive and negative ion modes.
In this work, we report the metabolite profiling using HPLC-MS in a QTOF, of a strain of *Fusarium oxysporum* that has significant antifungal activity against pythopathogens. After optimization of LC conditions such as different solvent systems and phase columns (C18, C8, PFP,HILIC) and in order to explore the whole universe of chemical structures that may be present in the extract, different sources were employed like ESI, APCI and APPI in both positive and negative ion modes. C18 chromatographic phase was selected for the analysis of the mycelium extract, PFP for medium analysis and HCOOH 0.1 % -MeOH as solvent system in both cases.
A complex metabolite pattern was observed in all LCMS runs. When ESI was employed, a greater number of metabolites were observed compared to APCI and APPI runs, while APCI and APPI gave similar results.
Using the obtained molecular formulas and MS/MS data, a search in different libraries and literature[1] was performed allowing the identification of seven cyclosporines, sansalvamide, *N*-methyl sansalvamide, a related cyclodepsipeptide not previously described, fusarubin and related compounds.
APCI and APPI resulted more sensitive for cyclic peptides, and MS/MS analysis of $[M+H]^+$ was especially useful for these compounds, which give predominantly sodiated forms in the ESI LCMS runs.
The use of negative ion mode allowed the identification of some compounds such as the antibiotic F244, where MS/MS fragments were very informative for its identification.
Many of the identified structures were confirmed by NMR spectroscopy after the isolation of the compounds.
Based on our previous results in the application of ESI-LCMS with post-column addition of metal solutions for the differentiation of isomers, as in the case of hydroxypyridine *N*-oxides[2] and dihydroxyarenes[3], we used this methodology to obtain further information about the metabolites produced by this fungus. The use of metal complexation by post-column addition of a solution of Ca (II) , Cu (II) or Zn (II) allowed the differentiation of isomeric Cyclosporine A from Isocyclosporine A, which are known to be indistinguishable by MS/MS[4]. In the MS spectra, complexes of cyclosporine A with the corresponding divalent metal were observed, whereas complexes were not observed for isocyclosporine A.
In conclusion, the use of different sources, ion modes and post-column addition of metal solutions allowed the complete analysis of the extracts of this fungal strain, providing complementary information.

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J. Am. Soc. Mass Spectrom. 2011, 22, 545-556.

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PWe-104
13:30 – 14:40

Pyruvate Phosphate Dikinase Phosphorylation Increases Lipid Production in Rhodopseudomonas palustris: a Phosphoproteomic Analysis

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Keywords:
Phosphoproteome, Rhodopseudomonas palustris, lipid production

Novel aspects:
Lipid production is regulated by protein phosphorylation which is identified by phosphoproteomic analysis

Abstract:
Rhodopseudomonas palustris (*R. palustris*) is a purple nonsulfur anoxygenic phototrophic bacterium with metabolic versatility. It is able to grow under photoheterotrophic and chemoheterotrophic states and has the potential for bioenergy production and biodegradation. This study is the first to identify the phosphoproteome of *R. palustris* including 100 phosphopeptides from 54 phosphoproteins and 74 phosphopeptides from 42 phosphoproteins in chemoheterotrophic and photoheterotrophic growth conditions, respectively. Among the phosphopeptides, phosphorylation at the threonine residue, Thr^{487}, of pyruvate phosphate dikinase (PPDK, *RPA1051*) was found to be involved in the regulation of carbon metabolism. Here, we show that PPDK enzyme activity is higher in photoheterotrophic growth, with Thr^{487} phosphorylation as a possible mediator. Under the same photoheterotrophic condition, bacteria with wild-type PPDKs showed enhanced accumulation of total lipids than those with the mutant (T487V) forms. This study reveals the role of the PPDK in the production of biodiesel material, lipid content, with threonyl-phosphorylation as one of the possible regulatory events during photoheterotrophic growth in *R. palustris*.

PWe-105
11:10 – 12:20

Rapid and Simple Characterization of Mycolic Acids in the Suborder Corynebacterineae Using MALDI Spiral-TOFMS

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Keywords:
Mycolic acids, Rapid and simple characterization, high mass resolving power, MALDI Spiral-TOFMS, Corynebacterineae

Novel aspects:
Crude mycolic acids in the suborder Corynebacterineae were successfully characterized using MALDI Spiral-TOFMS rapidly and easily.

Abstract:

Introduction

Mycolic acids are 2-alkyl 3-hydroxy fatty acids which are present in the cell envelope of the suborder *Corynebacterineae*, and used for their chemotaxonomy. Since compositions and contents of mycolic acids are related to virulence of *Mycobacterium tuberculosis*, their synthetic pathway is one of the targets for antitubercular drugs. As a result, the determination of the compositions and contents of mycolic acids is important. Mycolic acids in bacterial cells are classified based on the functional groups and the number of unsaturated bonds, and each mycolic acid has a distribution in the number of total carbon atoms. Though thin layer chromatography (TLC) methods have been used widely, it is impossible to analyze such complex mycolic acids without the help of other methods. Accordingly, to examine exact distribution of carbon chain length, each mycolic acid separated by TLC is further analyzed using MALDI-TOFMS. However, TLC methods are cumbersome so that it is not suitable for analysis of a large number of samples.

We expected that mycolic acids are analyzed using MALDI Spiral-TOFMS [1] without prior separation by TLC, because each mycolic acid with slight mass differences can be distinguished owing to high mass resolving power. In this study, rapid and simple compositional analysis of crude mycolic acids using MALDI Spiral-TOFMS was demonstrated.

Experimental

Mycolic acids were extracted from the genera *Corynebacterium, Dietzia, Rhodococcus, Gordonia*, and *Nocardia* by saponification with KOH followed by methyl esterification with methanol-toluene-sulfur acid. As the matrix for sample ionization, 2,5-dihydroxybenzoic acid was used. Samples were measured by the SpiralTOF (JMS- S3000 ; JEOL Ltd., Tokyo, Japan) , of which flight length was ca. 17 m along with eight cycles of the spiral trajectory to achieve high mass resolving power. The mass spectral data were analyzed using the Polymerix software (Sierra Analytics, Modesto, CA) .

Results

Many peaks of mycolic acid methyl esters were observed. Especially, in the MALDI mass spectra of the genus *Nocardia,* adjacent peaks originated from CH_2-CH_2 and C=O, whose mass difference is only Δ0.036 Da, were clearly separated thanks to the high mass resolving power of the MALDI Spiral-TOFMS. As a result, the number of total carbon and oxygen atoms, and unsaturated bonds were successfully obtained. Furthermore, relative peak intensity of each mycolic acid could be calculated. In this study, 16 categories based on 3-5 of oxygen atoms and 0-5 of unsaturated bonds were used for analysis of mycolic acids. These categories are related to the functional groups such as cyclopropane ring, methoxy group, and keto group. By showing the relative peak intensities of each mycolic acid in the contour map, in which horizontal axis indicates the number of oxygen atoms and vertical axis indicates the categories of mycolic acids, different distribution of mycolic acids in each strain were clearly visualized. This would be a new rapid and easy technique for the characterization of mycolic acids. This method could be applied not only for the chemotaxonomy, but also for the development of antitubercular drugs and vaccines.

Acknowledgment

This study was partly funded by a grant from the Institute for Fermentation, Osaka (IFO) .

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PWe-106
13:30 – 14:40

Evaluation of Proteomic Profile with Principal Component Analysis in Group A Streptococci

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Keywords:
Group A Streptococci, Shotgun proteome, Principal Component analysis, data-mining

Novel aspects:
We applied Principal Component Analysis (PCA) for shotgun-proteomic analysis of Group A Streptococci. The results suggested that the PCA is useful for data-mining of proteomic analysis.

Abstract:
Microbes modify its proteome to adapt the environment. It is well known that many pathogens produce exotoxins, cell surface proteins, or effectors to attach, survive, and spread in living host organisms. Therefore, the shotgun proteomic analysis is an effective approach to evaluate the phenotype of harmful infectious bacteria. The next challenge is to handling big data that derived from mass-spectrometer, and data mining to discover what proteins characterize the pathogenic phenotype. To harvest the fruitful information from proteomic profile, we applied the principal component analysis (PCA) to shotgun proteomic analysis of *Streptococcus pyogenes*. *S.pyogenes* is well known human pathogen that causing many infections including pharyngitis, impetigo, and toxic-shock like syndrome. This pathogen produces large amount of exotoxins such as proteases and superantigens. The proteome, including exotoxins, are influenced by culture conditions, however, the overall picture of changing proteome with growth phase have not clearly evaluated in *S. pyogenes*. Herein, we analyzed the proteomic profile with growth phase of *S. pyogenes*, and evaluated its profile with PCA.
In this study, the proteome of *S. pyogenes* SF370 was evaluated. The four point of cell culture were harvested during middle logarithmic to stationary phase under oxidatively (with shaking) or statc culture condition. The cell culture was divided into two subcellular fractions, supernatant (secreted proteins) , and whole cell fractions. The protein mixture was digested with trypsin, and the resulted peptides were served to the shotgun proteomic analysis. Detection of fragmented proteins are performed by LTQ- Orbitrap XL (ThermoFisher SceintificInc.) combined with Paradigm MS^4 LC system (MichromBioResources Inc.) .Obtained spectrum by LTQ is identified by MASCOT program based upon in-house amino acid database. To be identified, at least two unique amino acid sequences for each protein is required. Each of samples was measured in triplet for each of two subcellular fractions, four time points under two distinct incubation conditions. Analyzed quantity by PCA is percent of experimentally modified protein abundant index (%emPAI) .
As a result, 810 proteins were identified in two subcellular fraction, four time point and two (wish or without shaking) culture conditions. With the PCA analysis that 48 experiments (three subcellular fractions with two growth phases in triplet) were embedded into plane (D=2) , each experiments formed separate clusters. In first-round PCA, twenty-three proteins were picked up as the factors that characterized each experiment. After remove them, additional thirty proteins were picked up in second-round PCA. These picked up proteins were classified with functional category : translation (24 proteins ; including 20 ribosomal proteins) , carbohydrate metabolism (9) , chaperone (4) , amino acid metabolism (2) , replication (1) , energy production (1) , cell division (1) , signal transduction (1) , function unknown or prediction only (4) , and virulence associated proteins (6) . Furthermore, we tried to assign the biological meaning for these proteins. For example, peroxiredoxin reductase (SPy2079 : AhpC) , which is estimated to be involved in oxygen metabolism and hydrogen peroxide decomposition, was found in shaking culture condition rather than static condition. It seems reasonable that the increasing amount of AhpCin shaking condition because the shaking condition induces the higher oxygen stress. Another examples, many virulence associated proteins, pyogenic exotoxin B (SpeB ; SPy2039) , pyogenic exotoxin C (SpeC ; SPy0711) , mitogenic factors (Mf ; SPy2043, Mf 2 ; SPy0712, and Mf 3 ; SPy1436) , and M protein (Emm ; SPy2018) , are picked up by PCA analysis. SpeB increases with time-dependent, in both shaking and static culture condition. Both Mf 2 and SpeC increase under the shaking condition, but decrease under the static condition. The amount of both M protein and Mf increased, but that of Mf 3 decreased in shaking condition, although their amount keep constant value under the static incubation condition.
These results indicated that the PCA is useful for the data mining with proteomic analysis.

PWe-107
11:10 – 12:20

Glycopeptidolipids with novel peptide composition from Mycobacterium avium-intracellulare complex

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Keywords:
Mycobacterium, MAC, Glycopeptidolipid, GPL, Lipopeptide

Novel aspects:
We found novel glycopeptidolipids containing valine or leucine in the peptide core from Mycobacterium avium intracellulare complex. These structure were confirmed with MS, MS/MS and amino acid LC/MS analysis.

Abstract:

Introduction

Mycobacterium avium-intracellulare complex (MAC) is a widely distributed opportunistic pathogen that causes lung infections such as tuberculosis in immune-compromised hosts, and disseminated infections in AIDS patients. MAC specifically expresses glycopeptidolipids (GPLs) on their cell walls. GPL expression is strongly associated with colony morphology, sliding motility and biofilm formation.

In MAC, GPLs are commonly composed of a lipopeptide core structure, consisting of a peptide moiety (D-Phe-D-alloThr-D-Ala-L-alaninol), and a fatty acid. The lipopeptide is glycosylated with a 6-deoxy-talose to the D-alloThr and a rhamnose to the L-alaninol, respectively. This is called nonspecific GPL which is commonly expressed in all serotypes of MAC. This is further glycosylated with specific oligosaccharides at the 6-deoxy-talose. These are called sero-specific GPLs (ssGPLs), and have been serologically classified into ~30 species to date. Molecular diversity can occur on parts of the acyl moiety (chain length and methylation of hydroxy group) and sugar moieties (methylation and acetylation). These modifications influence host immune responses. Elucidation of the intact structure of GPL, including its molecular diversity is essential to understand MAC virulence.

We have studied the structure of ssGPLs using MALDI TOF-MS. When we applied total lipids of MAC serovar 4 to MS, we found two atypical ions of ssGPLs, which were 34 or 48 Da less than the dominant ion of ssGPLs. These ions could not be explained as resulting from conventional molecular diversity. Additionally, these atypical ions were also found in previous reports by other researchers using MAC. However, there have been no detailed studies performed on these ions to date. Therefore, we investigated the reason as to why these atypical ions appear.

Material and method

The reference strain of MAC was ATCC 700737 for serovar 4, and was cultured in Middlebrook 7H9 broth with oleic acid albumin dextrose complex enrichment (Becton Dickinson Co.) for 3 weeks. Whole lipids of autoclaved MAC were extracted using Folch's procedure. After alkaline methanolysis for eliminating acetyl groups from GPLs and other alkali-labile lipids, the alkali-stable lipids, including the GPLs, were recovered by Folch's procedure, followed by purification of deacetylated ssGPLs with the one-step column method. For the preparation of the lipopeptide component of GPLs, the purified deacetylated GPLs were subjected to acidic methanolysis. After that, the lipopeptide component was extracted by Folch's procedure.

MALDI TOF-MS and MS/MS spectra were obtained using an ultrafleXtreme (Bruker).

For preparation of amino acids, the lipopeptide component was subjected to acidic hydrolysis. The recovered amino acids were derivatized with 3-aminopyridyl-*N*-hydroxysuccinimidyl carbamate (APDS). The amino acids were analyzed by ESI-LC/MS using HPLC1100 (Agilent) and Esquire 3000plus (Bruker).

Results and Discussion

We observed ions at m/z 1671.9 and 1685.9, which were 48 and 34 Da less than m/z 1719.9, dominant ssGPLs ions of serovar 4, respectively. MS/MS spectra of these ions showed product ions such as rhamnosyl lipopeptide portion which kept the mass difference. Furthermore, MS/MS analysis of purified lipopeptide components showed that Phe was replaced by other amino acid.

LC/MS analysis of APDS derivatives of amino acids showed presence of Phe, allo-Thr, Ala, Val, Leu. However, we did not observe other amino acids. Considering these results, there were at least three lipopeptide species in the amino acid sequence, including Val-alloThr-Ala, Leu-alloThr-Ala and Phe-alloThr-Ala. Because Val, Leu and Phe closely resemble each other in hydrophobic property, mycobacterial peptide synthetase may incorporate these amino acids into GPLs.

We confirmed that mass spectra showing atypical mass differences were also observed in MAC serovar 2 (ATCC25291) and serovar 16 (ATCC13950) in our experiment. Therefore, Val- or Leu- containing GPLs are commonly expressed in MAC. However, it is unknown whether other *Mycobacterium* species that contain GPLs also express these atypical GPLs.

PWe-108
13:30 – 14:40

Identification of Medically Important Candida Species by LC-Selective Tandem Mass Spectrometry

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Keywords:
candida infections clinical LC-MS/MS markers

Novel aspects:
Detection of Candida species in a microbial mixture using a new approach - selective proteotypic-peptide analysis.

Abstract:
Candida infections are life threatening in neutropenic and AIDS patients. In particular, *C. albicans*, *C. glabrata*, *C. lusitaniae*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*, are the most frequently occurring species in the clinical practice. Therefore, accurate detection and species identification is of utmost importance to initiate appropriate therapy. Here we focused on reliable detection and identification of *candida* species present in a mixture by LC- selective proteotypic-peptide analysis method. The LC-MS/MS analyses of species-specific peptides and database search were used to accurately identify the fungal/microbial mixture. The differentiation/characterization of many *candida* species can be achieved by performing a series of MS/MS analyses of selected marker peptide ions. Furthermore, we managed to identify different *candida* species mixed in various abundances simultaneously during a single LC-MS/MS analysis.

Further, the complete genome of *C. krusei* is currently not available in NCBI protein databases (only 116 proteins are presented) . Therefore, we cannot use the database search algorithm to accurately identify proteins associated with *C. krusei.* To address this problem, we used an overlapping approach to find *C. krusei* biomarkers that are not unique to this species but are simultaneously present only in this species. *C. krusei* is considered present in the sample if these markers are all present in a sample and identified.

The method presented here (LC-selective MS/MS) provides the advantages of excellent selectivity and high accuracy for characterizing complex microbial mixtures. Also our findings indicate that the proposed approach could be an alternate in the near future of the traditional pathogenic identification methods.

PWe-109
11:10 – 12:20

Metabolome of Aspergillus: Towards the Early Diagnosis of Invasive Aspergillosis

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Keywords:
Aspergillus, Siderophore, Biomarker, Diagnosis, Aspergillosis

Novel aspects:
Focusing to metabolome in fungal diagnostics represents a similar benefit to what we have learned from proteomics : going from peptide mapping to peptide sequencing could give better identification rates.

Abstract:
Classification of microorganisms by MALDI profiling has already become a standard. With the growth of fungal databases, the remaining obstacles are represented by strain mix analysis, the longer cultivation period mandatory for sufficient fungal material production and presence of melanine-like structures interfering with MALDI analysis. At present, no data are available on the sensitivity of MALDI protein typing approaches of filamentous fungi including *Aspergillus*.
In this presentation we will report on development of highly specific molecular approaches for fast and early identification of aspergillosis, one of the most prevailing fungal infections occurring in immunocompromised humans. Targeted and untargeted metabolomic[1] and proteomic[2] approaches, utilizing combinations of separation and enrichment strategies, allowed us to define new small molecule and/or proteinaceous fungal biomarkers useful in early diagnostics, the analysis of mixed strains and fungal phenotype discrimination. In metabolomic approaches a special focus was dedicated to siderophores, mycotoxins, melanin structures, non-ribosomal peptides and lipids. For Fe-containing compound classification the negative mass defect and the $^{54}Fe/^{56}Fe$ isotope ratio data filtering was used.
Strain-specific low molecular weight biomarkers were identified in eight reference *Aspergillus* strains (including *A. fumigatus, A. flavus, A. niger*) using our in-house developed and publicly available software mMass[3]. The updated *Aspergillus* metabolite database was used for MALDI and NALDI-FTMS profiling data interpretation, as well as for fungal strain classification by HPLC-ESI/FTMS. In addition, a new *de novo* sequencing tool dedicated to non-ribosomal cyclic microbial products was also developed. In this software a large database of non-ribosomal peptide fungal building blocks was incorporated. The software did not only annotate and characterize the tandem mass spectrum of a known cyclic peptide, but also provided a sequence tag in an unknown natural product. We used the strategy of fragment ion "pre-characterization " based on its characteristic neutral losses and identification of the neighboring residues according to characteristic (pre-calculated) exact mass differences of one building block [or its di- (tri-) mers] . One unknown building block in the sequence did not necessarily hamper the sequence determination. The final sequences were scored by using comparison of the calculated theoretical peaklists (a- and b-ions, water and ammonia losses) with the acquired data.
We conclude that metabolomic approach conducted by mass spectral techniques provides not only the disease biomarkers but also may define new antifungal drugs. Siderophores being secreted in 8-12 hour time frame upon inoculation can serve as early diagnosis features also useful in ^{68}Ga-enhanced high-resolution computer tomography[4]. Biomarker enrichment strategies combined with advanced mass spectral software tools can help mass spectrometry to be soon translated from bench to bedside in small molecule area.

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PWe-110
13:30 – 14:40

Design Of Specific methodology For The Identification Of Beta-Lactamase Producing Enterobacteriaceae Using MALDI Mass Spectrometry

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Keywords:
Extended spectrum beta-lactamase, MALDI, sample preparation

Novel aspects:
The design of an algorithm and optimised sample preparation for the detection of beta-lactamase production in Escherichia coli.

Abstract:
The emergence of multi-drug resistant (MDR) enterobacteriaceae that can produce Extended Spectrum β-lactamases (ESBLs) is currently a major worldwide concern. They confer resistance to a wide spectrum of beta-lactam antibiotics currently used as 1 st line empirical therapy in the management of gram negative bacterial infections. *Escherichia coli (E. coli)* is the most frequent gram-negative bacillus isolated from blood cultures in clinical settings and *CTX-M-15bla* gene the most common coding for an ESBL worldwide. The use of MALDI mass spectrometry for microbial speciation is generally performed by MS only fingerprinting methods and is now widely applied. The technique is now standardized, but the commonly accepted methods do not allow differentiation between an ESBL producing *E. coli* and antibiotic sensitive *E. coli*. The aim of this study is to explore the variety of sample preparation methods inherent to MALDI and apply them directly to clinical/laboratory isolates in order to create a valuable diagnostic tool that can be used on an everyday basis in microbiology laboratories.

The isolates of *E. coli* usedcarry fully characterised plasmids with a variety of beta-lactamase genes under an inducible and controllable expression system. These isolateswere grown overnight on LB agar and a variety of extraction protocols and layering methods were employed. The utilisation of different layering methods led to changes in the crystallisation process. These were ultimately used in the optimisation of the resolving power of the generated superspectra. Several matrices were applied to these colonies as per standard protocol using layering. MS spectra were obtained on organisms using linear mode MALDI-TOF-MS (Axima™ Assurance, Shimadzu, Manchester, UK) .

An MS-only pattern recognition method using CHCA, sinapinic acid and DHB matrices was employed alongside machine learning using the SARAMIS™ software platform to create individual “superspectra “ for fully phenotypically and genotypically characterised isolates. “Superspectra “ created were then used in an attempt to identify both single and mixed populations of fully characterised *E. coli* isolates. Subsequently further *E. coli* isolates with full phenotypic and genotypic characterisations were submitted for comparison to the previously generated “superspectra “.

PWe-111

11:10 – 12:20

Differentiation of Single Attachment versus Double Attachment of Phycoerythrobilin Chromophores to Phycobiliproteins of Fremyella diplosiphon Cyanobacteria using MALDI Mass Spectrometry

M Nazim Boutaghou[2], Christina M Kronfel[2], Leanora S Hernandez[2], Avijit Biswas[2], Wendy M Schluchter[2], Richard B Cole[1,2]

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Keywords:
Fremyella, phycobiliproteins, phycoerythrobilins, MALDI, bilin-lyase

Novel aspects:
MS methods allow characterization of the site-specificity of cyanobacterial bilin lyases. A novel MALDI approach to distinguish between singly- and doubly-attached PEB chromophore-peptide complexes overcomes current MS limitations.

Abstract:

Introduction

Cyanobacteria use chromophorylated proteins called phycobiliproteins as light-harvesting structures. Bilin chromophore attachment to phycobiliproteins, an enzyme-catalyzed post-translational modification process, serves to improve the light absorption characteristics. Bilin-lyases attach a bilin chromophore to the phycobiliprotein through a thioether bond that covalently links the chromophore to a cysteine moiety of the phycobiliprotein. The tetrapyrollic bilin chromophores attach to the proteins through the 3^1 carbon of the bilin. Double attachment of the same bilin can occur as well, and in this case, carbons 3^1 and 18^1 of the bilin are each forming covalent linkages to distinct cysteine moieties on the phycobiliprotein.

State-Of-The-Art

Mass spectrometry has become a method of choice for investigating the site-specificity of cyanobacteria bilin-lyases. Mass spectrometric analysis of peptides generated by enzyme proteolysis enables the detection of proteolytic peptides containing bilin chromophores. Further analysis using tandem mass spectrometry allows localization of the specific site (s) of attachment by detection and identification of the peptide-linked chromophore. We have used this approach to characterize the site-specificity of newly developed bilin lyases CpeY & CpeZ, as well as CpeS. For the CpeY & CpeZ heterodimer, we were able to localize the site of PEB attachment to Cys-82 of the C-phycoerythrin alpha subunit, whereas CpeS attaches PEB specifically to Cys-80 of the C-phycoerythrin beta subunit. However, there is a mass spectrometric limitation when examining tryptic peptides containing two (or more) cysteines if one seeks to ascertain whether chromopeptides are singly or doubly attached. The problem is that singly and double attached chromopeptides appear at the same m/z value, thus, up until the present, only NMR analysis has been successful at determining the number of sites of chromophore attachment. Moreover, if the molecular weight of the peptide-chromophore complex is large, tandem mass spectrometry experiments relying on collision induced decompositions may be ineffective at generating fragments in high yields.

Novel Advance

We report in this work a new, fast and accurate method for discriminating singly from doubly attached chromophores using MALDI TOF/TOF mass spectrometry. This method was developed from mass spectral analysis of chromopeptides that had undergone *in vitro* and *in vivo* attachment of bilin chromophores to phycobiliproteins. Distinction is based on a difference corresponding to a characteristic neutral loss that appears in the MALDI-TOF mass spectrum only when the bilin is singly attached. When a tryptic peptide bearing a singly-attached PEB (covalently bound at the A-ring) is subjected to laser photons (λ = 355 nm) during a MALDI experiment, the C^{15}-C^{16} bond of PEB was observed to undergo a heterolytic cleavage which systematically results in the loss of the D-ring moiety of PEB. This cleavage reproducibly produces a fragment peak that is 121 Da below the mass of the intact protonated chromopeptide complex in the MALDI-ToF mass spectrum. In cases where the PEB moiety is doubly attached to the phycobiliprotein through the A- and D-rings, no such decomposition is observed. The appearance of a peak at 121 Da below that of the protonated tryptic peptide is deemed to be diagnostic of attachment of the A-ring of PEB at a single site on the phycobiliprotein. Conversely, failure to detect a peak at 121 Da below that of the protonated tryptic peptide is strong evidence of double attachment at the A- and D-rings of PEB. The validity of using the above-described photophysical property to identify the number of linkages is shown for singly attached PEB chromopeptides resulting from proteolysis of recombinant C-phycoerythrin alpha and beta subunits, singly attached PEB chromopeptides and a doubly-attached PEB chromopeptide resulting from the proteolysis of holo-phycoerythrin isolated from *Fremyella diplosiphon*.

PWe-112
13:30 – 14:40

The study and characterization of growth factors produced by murine herpes virus using multistage MS techniques

Andrea Stanova, Jozef Marak, Miroslava Supolikova, Livia Cikova, Frantisek Golais, Pavol Kois

Comenius University, Bratislava, Slovak Republic

Keywords:
mass spectrometry, murine herpes virus, growth factor, cellular transformation, separation methods

Novel aspects:
Detailed study of growth factor, its purification and physico-chemical characteristics along with its structure, using multistage MS techniques might open a new direction in research of herpes viruses.

Abstract:
At the present, the trends in modern analytical chemistry and biosciences are closely connected with the necessity to solve the complex analytical problems associated with multi-component qualitative and quantitative analyses of substances present in complex biological matrices at too different concentration levels (3-10 decimal orders). For successful solution of analytical problems associated with the analysis of substances present in complex matrices, one is always looking for new analytical approaches, often based on already known high-performance separation and detection techniques. Mass spectrometry (MS) is able to detect and identify analytes with high selectivity without a lost of sensitivity, dynamic range and/or speed of analysis. However, if the analyte is part of a complex mixture of substances, obtained mass spectra contain ions from all substances which are present at the same time in the ion source and, consequently, if the analyte is a minor component of the mixture, its identification with a certain degree of certainty is very difficult and in some cases mostly impossible. Taking the above mentioned facts in mind high performance liquid chromatography with mass spectrometry (HPLC-MS) took a significant and important position in bioanalytical chemistry.

There exist at least 8 strains of murine herpes virus (MHV), 5 strains (76, 78, Šumava. 4556, 5682) were isolated from Apodemus flavicolis, and 3 strains (60, 68, 72) were isolated from Myodes glareolus. A complete sequence and genome analysis is known only with MHV-68. It was shown later, that MHV-76 represents a naturally occurring mutant that lacks 9, 538 bp of the left end of the unique portion of the genome encoding nonessential pathogenesis-related genes. As concerns other isolates of MHV, they are only poorly characterized, if at all. Recently, some monoclonal antibodies (moabs) directed against gB glycoprotein of MHV-68 have been characterized, some of them, especially those recognizing N-terminal domain neutralize the virus, and some other give good reaction in immunoblotting or ELISA method. The use of these moabs might contribute to further characterization of all MHV isolates. Furthermore, there is known that herpes viruses are able to induce several types of persistent or latent infections and may be under certain conditions oncogenic. In studies of interaction between the various types of host cells and various herpes viruses a new class of substances resembling growth factors probably encoded by virus genome have been obtained. These factors are produced in certain cells in conditions non-permissive for virus replication, e. g. at supraoptimal temperature or in the presence of inhibitors of DNA replication, or in the cells which are naturally non-permissive for virus replication. Although these factors are only partially purified at present, their fundamental characteristics already exist. The cultivation of non-transformed cells in the presence of these factors leads to the appearance of the transformed phenotype, while the phenotype of transformed cells becomes changed towards the normal ones, As shown with the factor associated with human herpes simplex virus (HSV), the gB glycoprotein of this virus plays an important role in its synthesis and some moabs against gB of HSV neutralized the biological activity of this factor and the same has been shown with the factor associated with pseudorabies virus. Similar factor has been also demonstrated in cells infected with MHV-68.

This work was focud on detailed study of growth factor with the use of moabs against gB of MHV-68, its purification and physico-chemical characteristics along with its structure, using multistage MS techniques in combination with different separation techniques.

Acknowledgement : This work was supported by a grant from the Slovak Research and Development Agency (No.VVCE-0070-07) and the grants of Slovak Grant Agency, No`s.1/0265/11 and 1/1305/12.

PWe-113
11:10 – 12:20

MALDI Mass Spectrometry and MALDI-MS/MS Investigation into Chemically Induced Peptides of Beta-Lactamase Producing Enterobacteriaceae

Emmanuel Q Wey[2], Philippa J Hart[1], Matthew Openshaw[1], Omar Belgacem[1], Pranav Somiya[3], Shervanthi Homer-Vanniasinkam[3], Indran Balakrishnan[2]

[1]Shimadzu, UK, [2]Royal Free Hospital NHS Foundation Trust, London , UK, [3]NPIMR-UCL, London, UK

Keywords:
MALDI, Beta-lactamase production, sample preparation

Novel aspects:
The use of specific chemical cleavages prior to bio typing methods

Abstract:
The emergence of multi-drug resistant (MDR) enterobacteriaceae that can produce Extended Spectrum β-lactamases (ESBLs) is currently a major worldwide concern. ESBLs are a group of enzymes that are transferred via plasmids in gram negative bacteria. They confer resistance to a wide spectrum of β -lactam antibiotics currently used as 1st line empirical therapy in the management of gram negative bacterial infections. *Escherichia coli (E. coli)* is the most frequent gram-negative bacillus isolated from blood cultures in clinical settings and *CTX-M-15 bla* the most prevalent gene coding for an ESBL worldwide. The use of MALDI mass spectrometry for microbial speciation is generally performed by MS only fingerprinting methods and is now widely applied. The aim of this investigation was to use a bottom-up proteomics approach to highlight peptides/proteins associated with ESBLs, and consequently antibiotic resistance.

The methodology applied in this study deviates from MS-only pattern recognition methods. Here, isolate extracts underwent chemical cleavage, under optimised conditions using a variety of endopeptidases. MALDI-MS was used to generate polypeptide profiles thought to be related to the presence of β-lactamases in phenotypically and genotypically characterised isolates of *E. coli* carrying fully characterised plasmids with a variety of beta-lactamase genes under an inducible and controllable expression system. Tandem mass spectrometry was then used in an attempt to confirm the identity, and thus the relevance, of these peptides.

E. coli strains were grown overnight on LB agar prior to extraction. *E. coli* carrying fully characterised plasmids with a variety of beta-lactamase genes were identified using linear mode MALDI-TOF-MS (Axima™ Assurance, Shimadzu, Manchester, UK) and SARAMIS™ software. Once identity was confirmed, the β-lactamase containing *E. coli* and antibiotic sensitive controlswere subjected to chemical cleavage, and co-crystallised with matrices under varying conditions. *In-silico* digests and blasts of the β -lactamase enzyme were performed via the Expasy Bioinformatics Resource Portal. The peptides generated *in-silico* were then searched for in the peptide mass fingerprints generated as above. Subsequently, any *m/z* species that were found to be present in both the theoretical and experimental data were subject to MALDI-MS/MS. All MS/MS data were submitted to MASCOT™ for peptide/protein identification. Antibiotic sensitive *E. coli* was analysed and processed alongside ESBL containing *E. coli* to confirm the specificity and relevance of the possible peptides/proteins detected. It is hoped that this workflow will allow for the reproducible identification of peptides/proteins that are truly representative of ESBLs and as such, may be applied as markers for the purpose of rapidly determining the B-lactamase profile of *E. coli*.

PWe-114 Exploring the Patellamide Biosynthetic Pathway

13:30 – 14:40

Shirran L Sally[1], Fuszard Matthew[1], Koehne Jesko[1], Bent Andrew[1], Houssen Wael[2], Jaspars Marcel[2], Botting H Catherine[1], Naismith H James[1]

[1]University of St Andrews, [2]University of Aberdeen

Keywords:
patellamide, cyclic peptide, MRM, biosynthetic pathway, MALDI

Novel aspects:
Biosynthetic pathway dissection, novel substrate utilization to produce products of unknown biological activity, tailoring enzymes to produce novel cyclic peptides containing heterocycles

Abstract:
The patellamide cyclic peptides have been shown to have biological activities which include cytotoxicity and the ability to reverse multiple drug resistance in human leukaemia cells by antagonizing the p-glycoprotein export of vinblastine[1]. They are synthesized ribosomally as a pre-propeptide (PatE) , which is then tailored by a series of enzymatic (and possibly non-enzymatic) chemical transformations to produce a modified cyclic octapeptide, containing oxazoline and thiazole heterocycles derived from serine and cysteine[2]. We are using a mixture of mass spectrometric, protein X-ray crystallographic and biochemical methods to elucidate the biosynthetic pathway by characterising the individual enzyme products and, hence, tease out the mechanistic details of the transformations. We aim to exploit any flexibility in the enzymes' substrate tolerance to produce novel cyclic peptides, which may have interesting biological activities. We have developed a range of mass spectrometric methods, utilizing both MALDI (ABSciex 4800 TOF/TOF Mass Analyser) and ESI (Waters LCT, ABSciex QTrap 4000 and ABSciex TripleTOF 5600) instruments, to follow the individual steps in the biosynthetic pathway. MRM analysis delivers sensitive detection of signature MS/MS transitions in a complex background and hence allows us to follow the fate of specific functional groups in order to determine the products of the enzyme reactions and monitor enzyme turnover. Also presented are the MSMS fragmentation patterns observed for our cyclic peptides.

1. Houssen and Jaspars, 2010, Chembiochem, 11, 1803-15.
2. Schmidt et al., 2005, Proc Natl Acad Sci U S A, 102, 7315-20.

PWe-115
11:10 – 12:20

Liquid chromatography-electrospray ionization mass spectrometry for metabolism studies of mogroside V by Ganoderma lucidum mycelium

Chun-Hui Chiu, Ting-Jang Lu

National Taiwan University, Taipei, Taiwan

Keywords:
Siraitia grosvenorii, Ganoderma lucidum , LC-MS, mogroside, metabolism

Novel aspects:
The enzymatic system has a potential for mogrosides transformation, owing to its high specificity, yield, and productivity, and this method could be apply to the structural modification and metabolism studies.

Abstract:
The sweetness of *Siraitia grosvenorii* (Lo Han Kuo) fruit comes from a mixture of triterpenoid glycosides, mogrosides I-V, with mogroside V as the major component. Mogrosides have demonstrated blood glucose regulating activity. The purpose of this study was to investigate the structural conversion of mogroside V by *Ganoderma lucidum* mycelium. The metablolites of mogroside V were analyzed by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry and NMR spectrometer. The mogroside V molecule sequentially lost its glucosyl unit as the mass spectrometry being operated at collision-induced dissociation mode. Adding 1 or 2 % of water extract of the fruit did not impact the growth of mycelia in a malt extract medium. During the growth of the *G. lucidum*, the mycelium showed significant activity of beta-glucosidase and able to hydrolyze the glucosyl residues of mogroside V. The major metabolite of mogroside was tritepene triglucoside, Mogroside III E. The mycelia of *G. lucidum* were able to further utilize one glucosyl residue on Mogroside IIIE and converted it to triterpene diglycoside, Morgroside II A when the carbon source was limited. According the results of high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry, the beta- (1,2) linkage of glucosyl were more resistant to hydrolysis of beta-glucosidase of *G. lucidum*.

PWe-116 Identification of Bacteria and Fungi by Rapid Evaporative Ionization Mass Spectrometry

13:30 – 14:40

Ákos Szekeres[1], Imola Körtvélyessyné Györi[2], Júlia Balogh[2], Tamás Szaniszló[3], Katalin Dr Kristóf[3], Judit M Dr Molnár[3], Zoltán Dr Takáts[4]

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Keywords:
REIMS, Indentification, Bacteria,

Novel aspects:
Novel mass spectrometric method for rapid identification of microorganisms without sample preparation direct from the agar plate.

Abstract:
The rapid identification of microorganisms has a great importance in routine clinical investigations, by aiding the fast commence of adequate antimicrobial treatments. Using mass spectrometric (MS) techniques, characteristic spectral fingerprint of bacterial cells is obtained in a short time frame, which provides chemotaxonomic information as a basis for identification. Matrix Assisted Laser Desorption (MALDI) ionization is already implemented at the routine level for the rapid screening of unknowns, replacing classical approaches and carbon source utilization tests. Intact cell MALDI allows a relatively fast microbial identification of isolated colonies, however its application also requires some level of culturing, which dramatically increases the overall time demand of identification. A fast and cost effective technique for exact identification of bacteria and/or fungi would avail rapid introduction of adequate therapies.
In our study we assessed the possibility of mass spectrometric identification of different microorganisms by the use of the newly developed Rapid Evaporative Ionization Mass Spectrometry (REIMS) using a LTQ linear ion trap mass spectrometer. REIMS spectra of bacterial cells similarly to that of mammalian tissue samples features predominantly cellular membrane forming polar lipid components. These molecules are present in all examined organisms and possess a proper heterogeneity for species- or strain-level identification and differentiation. During the examinations nearly 2000 well defined clinical isolates belonging to 20 different genera were tested, which were precultured for 24 hours on three types of diagnostically used agar media ; blood, Mueller Hinton and chocolateagar. REIMS analysis of the fingerprinted molecules was carried out by evaporating bacterial biomass using high frequency electric current. Modified electrosurgical bipolar forceps were employed as an atmospheric pressure ion source producing gaseous ions of certain bacterial cell components. Aerosol formed on the rapid evaporation of bacterial cells was transferred to mass spectrometer using a Venturi air jet pump. It is important to note, that the whole experimental setup was microbiologically separated from the working environment with an isolation box design arrangement. The mass spectra were collected in single stage MS, negative ion mode, in the mass range of 600-900 *m/z* at unit mass resolution. The obtained spectra of replicate analyses of strains on different media were compared and found highly specific and characteristic for the species analyzed. Clinical isolates with similar taxonomical classification grouped well using principal component analysis, while individual groups showed complete separation using the first 3 principal components.
Identification method for unknown isolates was developed, based on the combination of principal component analysis and linear discriminant analysis. Authentics trains obtained from ATCC were analyzed by REIMS, and resulting data was used as a learning set. Learning set data was subjected to dimension reduction by PCA, and first 60 PC was subjected to LDA. Data points corresponding to unknowns were localized in the 60 dimensional LDA space and were classified into closest strain-specific data group. The method was proven to be insensitive for any examined perturbation, including colony age or substrate type.
The accuracy of identification procedure was higher than 99 % achieved using the PCA/LDA algorithm and aspectral database collected from ATCC strains.

PWe-117
11:10 – 12:20

Lysosomal storage diseases diagnostics in dry blood spots by fluorimetry and multiple reaction monitoring mass spectrometry

Claudia Cozma[1], Marius-Ionut Iurascu[1], Laura Ion[1], Stefan Maeser[2], Michael Przybylski[1]

[1]University of Konstanz, Germany, [2]Centogen GmbH, Germany

Keywords:
Lysosomal storage disease, diagnostic, multiple reaction monitoring

Novel aspects:
Novel working aproach for the deternimation of lysosomal ezymes by fluorimetry mass spectrometry

Abstract:
The loss of lysosomal enzyme activity is a characteristic of lysosomal storage diseases (LSDs) , a group of mostly genetic metabolism (e.g., α-galactosidase Fabry's Disease ; β-glucocerebrosidase Gaucher's Disease ; α-L- iduronase mucoplysaccharidose I) . The enzyme substrates can no longer be processed and are accumulating in the lysosome causing severe disease symptoms leading to multiple organ failure and finally death. For Fabry's Disease (FD) enzyme replacement therapy is available from 2001 with high success, thus rendering rapid and efficient diagnosis of key importance. In the present study we have developed and established specific and highly sensitive diagnostics tools using mass spectrometry multiple reaction monitoring approach (MS-MRM) . A fluorometric determination for monitoring of α-galactosidase, β-galactosidase and β-glucuronidase activity was developed as control assay. A clinical diagnostic study was performed using the dry blood spot (DBS) method with samples from ca. 100 healthy controls and Fabry patients. Tandem mass spectrometry offers a qualitative dimension by identification of the product, but also a quantitative dimension by introducing in the reaction an internal standard. The novelty of the present assay is the adaptation of the working conditions for enzymatic determination for the mass spectrometers used, reducing the time for sample preparation and also the introduction of MS/MS diagnostic spectra. The MS-MRM diagnostics of FD was carried out using a special mixture of substrate and internal standard. Substrate used used for enzymatic determination of alpha-galactosidase is (6-benzoylamino-hexyl) -{2- [4- (3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-xyloxy) -phenylcarbamoyl] -ethyl}-carbamic acid tert-butyl ester (SGLA) ($C_{33}H_{47}N_3O_{10}$; MW 645.7Da) and the expected product is (6-benzoylamino-hexyl) - [2- (4-phenylcarbamoyl) -ethyl] -carbamic acidtert-butyl ester (PGLA) ($C_{27}H_{37}N_5O_5$; MW 483.6 Da) . The internal standard for qualitative monitoring of the reaction was deuterated PGLA ($C_{27}H_{32}N_5O_5D_5$; MW 488.6 Da) . Diagnostic spectrum was established for the product and the internal standard for identification, and the concentration of both were monitored by MRM (multiple reaction monitoring) . Using the relative intensity of the peaks and knowing the concentration of the internal standard, the concentration of the product was evaluated using an equation characteristic for each mass spectrometer. Atypical healthy control sample in the MRM- MS assay gives an average substrate conversion of 6.6 mM pro liter per 48h (or 2,13nmols/spot/48h) . A DBS sample collected from male FD patient presents an activity 7 to 18x less (under 0,28 nmols/spot/48h) .

PWe-118
13:30 – 14:40

UV action spectroscopy of peptides and proteins via photodissociation of iodine labelled tyrosine residues.

Benjamin B Kirk[1], Adam T Trevitt[1], Haibo B Yu[1], Yuanqi Tao[2], Benjamin N Moore[2], Ryan R Julian[2], Stephen J Blanksby[1]

[1]University of Wollongong, Wollongong, Australia, [2]University of California, Riverside, USA

Keywords:
Action Spectroscopy, Peptides

Novel aspects:
A method of measuring ultraviolet action spectra of peptides and proteins using selectively iodinated tyrosine residues.

Abstract:
The advent of soft ionisation techniques such as electrospray ionisation, coupled with its unprecedented speed and sensitivity, has imparted on mass spectrometry an important role in the structural characterisation of peptides and proteins. The three-dimensional structure of proteins directly affects their biological activity. The capability to ionise and transfer both small peptides and large proteins intact into the gas phase provides a versatile tool with which to interrogate these molecules. While ion spectroscopy is an attractive means of directly probing the structure of these molecules, the low concentrations employed, and difficulty in gaining unrestricted access to the trapped ions, means structural characterisation is typically carried out by action spectroscopy. Instead of measuring absorbance and fluorescence directly, action spectroscopy is a measure of ion fragmentation yield relative to excitation wavelength.

Unfortunately, as molecular weight increases so does the energy required to fragment an ion. Intramolecular vibrational redistribution (IVR) results instead of direct bond cleavage, as any energy imparted on the ion is delocalised from the absorbing chromophore and redistributed into vibrational degrees of freedom. To overcome this limitation, one may instead measure fast dissociation phenomena, such as electron photodetachment, as recently employed by Dugourd and co-workers. [1] While an effective technique for measuring multiply charged anions, this method cannot always be used to measure singly charged anions or ions that are positively charged. An alternative is to introduce a functional group that dissociates prior to IVR. Recent work has demonstrated that peptides and proteins containing selectively iodinated tyrosine residues dissociate promptly on irradiation with a 266 nm laser pulse, generating an iodine atom and a carbon centred radical. Evaluating this dissociation as a function of wavelength affords a powerful, extensive method of measuring structural changes in a peptide or protein using action spectroscopy.

In these experiments, a Thermo Fisher LTQ linear quadrupole ion trap mass spectrometer has been modified to allow irradiation of trapped ions with a tunable laser pulse. The laser is a frequency-doubled OPO pumped by the third harmonic (355 nm) of a Nd : YAG laser, providing a tunable wavelength range between 220 - 350 nm. Coupling the Thermo Fisher Xcalibur analysis software with custom LabView scripts enables a simple, semi-automated process to measure action spectra at nanometre resolution.

In this presentation, we introduce iodinated tyrosine residues as target chromophores for action spectroscopy and outline an investigation of the charge-state modifications of small iodine labelled peptides and proteins. Action spectra of several charge-states of 3-iodotyrosine, iodinated KGYDAKA and ubiqituin are reported and a comparison made to the reported three-dimensional structures of these molecules measured by UV absorption and electron photodetachment yields in solution and the gas phase, respectively.

[1] Bellina *et al.* ; *Int. J. Mass. Spectrom.* (2010) ,**297**,36.

PWe-120 Influence of end-capping reagent of LC column on sensitive determination using LC/MS

13:30 – 14:40

Chiaki Aoyama, Yuko Yui, Kensuke Okusa, Kosuke Osaka, Takao Tamura, Masakazu Takahashi

GL Sciences Inc., Iruma, Japan

Keywords:
LC/MS, quantification, sensitivity, peak shape

Novel aspects:
Reagent used for end-capping often influences on sensitivity, and electrically neutral column is most recommended for highly sensitive analysis.

Abstract:

Introduction

LC/MS is a powerful and absolutely necessary technique for analysis of organic compounds. LC columns, which are used to separate target compounds from interfering material, play an important role as ion source, mass analyzer, and detector.

The most widely used columns for LC/MS are ODS columns. End-capping is exceptionally important for ODS columns because it greatly influences on peak shape. If tailing or leading occurs, peak height is diminished and sensitivity is lowered. Therefore, most of commercially available LC columns are end-capped with a variety of reagents.

In this study, LC columns end-capped with several reagents are compared. Peaks shape and sensitivity of drug compounds were compared in our poster.

Material and method

LC/MS system used in this study was consisted of an LC800 liquid chromatograph (GL Sciences) and a 4000QTRAP mass spectrometer (AB Sciex) . Isocratic or gradient elution was carried out using 0.02% formic acid aqueous solution as mobile phase A and acetonitrile as mobile phase B.

Trimethylchlorosilane (TMCS) , hexamethyldisilazane (HMDS) , and trimethylethoxysilane (TMES) were used as end-capping reagent and compared. Same silica gel to which octadecylsilyl group was chemically bonded was reacted with each reagent.

Procainamide, ranitidine, acetaminophen, caffeine, chlorpheniramine, propranolol, ketoprofen, salicylic acid, and carbazochrome sulfonate were chosen as analyte. Salicylic acid and carbazochrome sulfonate were detected in negative mode, while the others were in positive mode.

Preliminary results and discussion

TMCS and HMDS have frequently been used as end-capping reagent. However, it is known that residual hydrochloric acid and ammonia, respectively, exist on the surface of silica gel after the end-capping reaction. TMES was also selected in this study because we considered that electrostatically charged by-product should not be yielded by end-capping with this reagent.

Firstly, peaks of chlorpheniramine, which is a basic compound, obtained with each column were compared. The compound was eluted with good peak shape from HMDS column and TMES column. However, peak tailing occurred and S/N ratio was decreased when TMCS column was used. It was thought to be caused by partial adsorption owing to electrostatic interaction between positive charge of the analyte and negative charge of residual chloride ion on the surface of the silica gel.

Secondly, retention times of procainamide, which is a basic and highly hydrophilic compound, were compared. Although the compound was retained on TMES column to some extent, almost no retention was observed on HMDS column. We considered that its retention on HMDS column was prevented by electrostatic repulsion between positive charge of the analyte and that of by-product of HDMS end-capping. HMDS column is not desirable for determination of procainamide because its peak should be susceptible to ion suppression caused by unretained material.

As for carbazochrome sulfonate, significant adsorption occurred only when HMDS column was used. It can also be explained by interaction between negative charge of carbazochrome sulfonate and positive charge of HMDS.

In conclusion, only TMES column provided good peak shape and retention for all compounds examined in this study. LC columns which do not bear any charge on the surface of silica gel, such as InertSustain C18, are most recommended as first choice column for highly sensitive determination using LC/MS.

Poster Session

Wedensday, 19th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 29: The Advances in Biological Mass Spectrometry in Drug Discovery and Development: Current State of the Art and Challenges

PWe-121

11:10 – 12:20

Simultaneous, Fast Analysis of Melamine and Analogues in Pharmaceutical Components Using Q Exactive - Benchtop Orbitrap LC-MS/MS

Kate J Comstock, Hongxia (Jessica) Wang, Tim Stratton, Yingying Huang

Thermofisher Scientific

Keywords:

Benchtop-Orbitrap. Identification, Quantification. Melamine. Pharmaceutical

Novel aspects:

Novel approach - simultaneous, fast analysis of melamine and analogues in pharmaceutical components by high resolution benchtop Orbitrap LC-MS/MS

Abstract:

Potential drug contamination by melamine and its analogues remain a major concern by FDA. We present a workflow for analysis of melamine and its analogues in at-risk pharmaceutical components using high resolution benchtop Orbitrap LC-MS/MS : Q Exactive. Simultaneous, fast screening and quantitation for melamine and its analogues in complex matrices were achieved by HRAM full scan, ms/ms in a data-dependent fashion with polarity switching. Comparing with commonly used Triple Quadrupole MS method, this workflow allows for added post analysis flexibility, it avoids the upfront selection of specific compound masses pertaining to SRM methods. Confident identification is achieved by accurate mass measurement of both precursor and fragment ions, as well as the fine isotope pattern of 13C and 15N.

Methods

A calibration curve, containing melamine, ammeline, ammelide, and cyanuric acid, was prepared by serial dilution of a 1 mg/ml stock solution to give a final concentration range from 25ppb to 10ppm. Sample preparation recovery was determined by spiking neat solutions into the commercial pharmaceutical excipients tested in this study. Q Exactive coupled to Ultimate 3000 UHPLC system (Thermo Scientific) was employed for the studies. LC base line separation of all four compounds was achieved within 5 minutes using Thermo Accucore HILIC column. Full scan MS at resolving power 70,000 FWHM and MS/MS at resolving power 17.500 FWHM were collected in a data-dependent fashion with polarity switching. The US FDA method for melamine and cyanuric acid was referenced with modifications.

Preliminary Data

Initial data showed that full scan analysis at 70,000 resolution (FWHM) provides spectra of high quality that have sub- 2 ppm mass accuracy with external calibration and well-separated isotopic pattern of A+ 2 isotope ion on 13C and 15N. The high resolution accurate mass measurement, fine isotope pattern and signature product ions provide a multi-dimensional confirmation for melamine and its analogues analysis. Product ions from MS/MS spectra were utilized for quantitation. The results have good linearity on five-point calibration curves over the range from 5 ppb to 50ppm with excellent linear regression coefficients ($r^2 > 0.99$) and above 85% recovery rate. The preliminary data suggested that this robust UHPLC- HR MS/MS method provides simultaneous, simple, fast determination and quantitation of melamine and its analogues at a high confident level in a variety of pharmaceutical components.

PWe-122

13:30 – 14:40

Measurement and Optimization of Organic Chemical Reaction Yields by GC-MS with Supersonic Molecular Beams

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Tel Aviv University

Keywords:
GC-MS, Cold EI, Chemical Reaction,

Novel aspects:
New method and GC-MS instrument for the measurement and optimization of organic chemical reaction yields. GC-MS with enhanced molecular ion, extended range of analysis and uniform response

Abstract:
Chemical reactions are typically performed via mixing reactants together in an appropriate solvent, often with a catalyst, and allowing them to undergo a reaction for a few hours. At the perceived end of the reaction, the products are separated and purified, a process which could take several days. Subsequently, the purified products are analyzed by NMR and high resolution ESI-MS (typically with HR-QTOF) . However, no information is obtained about the synthesis yield, products purity, availability of isomers and on the reaction mechanism. GC-MS with its standard electron ionization, can provide such information but only for volatile compounds that exhibit molecular ions. Thus, a new MS system is needed to enable semi-on-line, monitoring of the reacting compounds and products in organic reactions.
Measurement and optimization of chemical reaction yields was performed by our Aviv Analytical 5975-SMB GC-MS with supersonic molecular beams (SMB) . It is based on the coupling of SMB interface and its fly-through electron ionization ion source with an Agilent 7890 GC + 5975 MSD. The GC eluting molecules are mixed with helium make up gas, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, collimated into a SMB, pass a fly-through electron ionization ion source where they are ionized with uniform response and mass analyzed. The vibrational cooling enhances the molecular ions and the combination of short columns and high column flow rates about doubles the range of thermally labile and large molecules amenable for analysis.
We used our 5975-SMB GC-MS with SMB for semi-on-line monitoring of synthetic organic chemical reactions for their reaction yield optimization, mechanism elucidation and for obtaining information on the reaction products identity, purity and availability of isomers. Several different organic synthetic reactions were studied and will be described and demonstrated. These reactions included the synthesis of a $C_{14}H_{18}N_2O_3S_2$ potential anti fungal drug compound (MW=362) , a $C_{25}H_{25}NO_4S$ compound (MW=435) which is targeted for chemical sensing of explosives, a $C_{50}H_{96}N_2O_8$ compound (MW=852) which is aimed at environmental metal remediation and the oxidation reaction of few thioesters.
The key features of the 5975-SMB GC-MS with Cold EI (EI of vibrationally cold molecules) that uniquely enabled such measurements are :
A) The fly-through EI ion source provides degradation and tail-free close to uniform compound independent response, thereby enabling the measurement of reaction yields from total ion count peak areas while using the standard Chemstation percent area report.
B) The molecular ions are enhanced and are practically always observed.
C) Isotope abundance analysis automatically inverts the molecular ions into elemental formulas.
D) The use of short columns and high column flow rates significantly extends (~doubles) the range of thermally labile and low volatility compounds amenable for analysis.
E) The high total ion count signal to noise ratio obtained enables the identification and quantification of both major and minor by-products and impurities
F) The chromatography measurement time is much faster than with standard GC-MS, from one minute to several minutes analysis cycle time.
Based on the collected data, we were able to better understand how the reaction conditions should be optimized in order to maximize the yields and purity of target products. Consequently, we propose that GC-MS with SMB can serve as a novel tool for the optimization of chemical reactions in organic chemistry departments, drug synthesis and in the chemical industry.
Our goal is to advance towards a change in the way in which organic chemistry is being performed and eliminate the lengthy steps of post reaction sample separation and purification while enabling reaction yield optimization and product purity improvement.

PWe-123
11:10 – 12:20

The Utility of a Novel High Resolution Quadrupole Time-of-Flight Mass Spectrometer for Improved Characterization of Antibodies and Immuno-Conjugates

Asish Chakraborty[1], St John Skilton[1], Steve Pringle[2], Nick Tomczyk[2], Weibin Chen[1]

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Keywords:
Antibody, ADC, Antibody-drug conjugate, SEC-MS

Novel aspects:
Sensitive and high resolution MS characterization of immuno-conjugates using high-throughput on-line size exclusion chromatography coupled directly to electrospray ionization mass spectrometry

Abstract:

Introduction: Monoclonal antibodies (mAbs) and derivatives for the treatment of cancers and inflammatory diseases are a rapidly growing class of biotherapeutic. mAbs are often combined with cytotoxic drugs to enhance their therapeutic efficacy, and alternatively, small anti-neoplastic molecules can be chemically conjugated to mAbs. Typically, several molecules of a potent cytotoxic-agent are covalently linked to a MAb, and therefore structural characterization is challenging due to the heterogeneous nature of the conjugate, the large molecular-size of antibodies and low abundances of the modified residues. Additionally immunoconjugates might differ in number of drugs linked and the sites of linkage. Here, we present a high-sensitivity quadrupole/ time-of-flight system with novel ion-guide source for characterization of mAbs and conjugates that helps unravel some of the complexity.

Methods: Intact and deglycosylated-conjugates, and naked antibodies were introduced to the ESI mass spectrometer using two different chromatographic conditions : either a generic size-exclusion chromatography (SEC) with high-throughput MS-friendly conditions, or high-resolution reversed-phase chromatography (RP) method. The naked antibody, conjugate, and their deglycosylated forms were also reduced with DTT to the light chain and heavy chain fragments and were separated on a SEC column with sub- 2 micron particles, and analyzed using a prototype quadrupole/ time-of-flight system equipped with an off-axis conjoined stacked ring ion guide. RP separation was also used to separate the naked and conjugated-antibody light and heavy chain fragments and to automatically determine the conjugation sites by peptide mapping.

Preliminary Data: Antibody drug-conjugate analysis is more complex than the naked antibodies. The use of MS-friendly SEC with sub- 2 micron column packing materials, directly coupled to a quadrupole time-of-flight mass spectrometer for the mass analysis of antibody drugs and conjugates provides improved detail and productivity than previous generations of instrument. Light and Heavy chains were baseline separated for all antibody and conjugate samples. SEC, using aqueous 30% acetronitrile with acidic modifiers as mobile phase, directly coupled to ESI-MS provided a routine analysis method for profiling of antibodies and conjugates, degree of conjugations, sequence confirmation (isotope resolution) , and molecular-weight measurement.

Intact subunits of mAb and conjugates from DTT reduction were separated by high-resolution RP chromatography which resolved different isoforms coexisting in the sample. High-resolution MS detection was able to istopically resolve light chain (25 kDa) . Identities of either intact mAbs or conjugates, or the subunits were automatically confirmed by deconvoluting the mass spectra using both maximum entropy and BayesSpray deconvolution algorithms. The deconvoluted mass spectrum of deglycosylated-conjugate shows several peaks with approximately same mass differences and with varying intensities. The masses of these peaks are assigned to naked mAb and to mAb conjugates with varying number of covalently linked drug-molecules. The deconvoluted MS spectra of deglycosylated reduced mAb-conjugate show both light and heavy chains are modified and conjugated with drugs. The total number of linkers attached to the light and heavy chains agrees with the number of linkers attached to the deglycosylated intact conjugate. The LC-TOF MS approach presented here facilitates rapid characterization of antibody conjugates. We are currently working on detecting the conjugation sites using trypsin/AspN peptide mapping

PWe-124

13:30 – 14:40

Method Development for Quality Control of Traditional Chinese Herbal Medicine- Guizhi Tang by HPLC and Electrospray Ion Trap Mass Spectrometry

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Keywords:
Guizhi Tang ; Ion Trap ; Mass.

Novel aspects:
New LC/MS analysis method of the Chinese Medicine prescription- Guizhi Tang.

Abstract:

Introduction:
Traditional Chinese Herbal Medicine (TCM) is utilized regularly in the multi-herb prescription for treating people's disease. The quality control of the prescription and their derived products is more difficult than the single herb. In this study we have used LC/ion trap MS analysis to develop a simple and highly efficient analysis method for the Chinese Medicine prescription- Guizhi Tang.

Methods :
An extract PDC-1689 as Guizhi Tang was prepared from traditional Chinese herbal medicines containing *Ziziphus jujuba* Mill, *Zingiber officinale* Rosc, *Paeonia lactiflora* Pall., *Glycyrrhiza glabra* L., and *Cinnamomum cassia* Presl (material ratio as follows, 5 : 6 : 6 : 4 : 6) . PDC-1689 was analyzed by Agilent1100 high performance liquid chromatography (Agilent Technologies, CA, USA) , Brukeresquire HCTion trap mass spectrometry (Bruker, Bremen, Germany) equipped with Agilent 1100 HPLC, and Nuclear Magnetic Resonance Spectroscopy (Bruker, Bremen, Germany) .

Results and conclusion:
Different elution conditions of reversed-phase HPLC for PDC-1689 assay were developed and optimized. The optimal conditions were found of C-18 reversed phase column with a mobile phase of 0.05% Formic acid and Acetonitrile at a flow rate of 0.8mL/min. UV detection was achieved at 254 nm. Under the optimal conditions, seven compounds with retention time of 21.2, 23.2, 24.1, 26.1, 32.9, 64.9, 72.6 min were analyzed by electrospray ion trap mass spectrometry with positive-ion mode at *m/z* 563.1, 551.1, 431.0, 839.4, 823.4, and negative-ion mode at *m/z* 833.5, 510.8. The structures were elucidated by LC-SPE-NMR and led to the identification of glycyroside, isoliquiritin apioside, formononetin 7-*O*-glucoside, isoliquiritin, liquiritigenin, licorice-saponin G 2 and glycyrrhizic acid.

PWe-125

11:10 – 12:20

High-throughput sequence determination of screened 90 micron single beads on which a cyclic octa-peptide is immobilized

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Keywords:
HTP-sequencing, cyclic-peptide, PEG-based resin, OPOB

Novel aspects:
High throughput sequencing for cyclic peptides immobilized on PEG-based beads, screened by assays in aqueous media, had been developed. The method involves partial hydrolysis followed by nano-LCMS without encoding technologies.

Abstract:
Peptide library constructed using the concept of "one peptide immobilized on a single bead (OPOB)" is useful for the discovery of interacting molecules [Lam KS, Salmon SE, Hersh EM (1991) Nature 354：8284]. In particular preliminary information regarding ligands can not be obtained. While gel-type polymer beads are often used as supports, peptide assembly is carried out in organic solvents, although assays are performed in aqueous media. Hence library construction is often performed on polyethylene glycol (PEG) based resins. Sophisticated encoding technologies such as DNA or small functional groups followed by deconvolution using DNA-sequencer or GC-MS have been carried out [Still WC (1996) Acc. Chem. Res. 29：155-163], although the simplest deconvolution method is the Edman degradation using a peptide-sequencer [Liu R, Lam KS (2001) Analytical Biochemistry 295：9-16]. However the Edman method has restrictions as relatively larger amounts (tens of picomoles) of sample and N-terminal free amino groups are required. Therefore N-terminal amino acid should not be blocked during screening/assays. Additionally the Edman cycle is time consuming since the determination of amino acids is performed on a one-residue basis and thus costly. To overcome these restrictions we have attempted top down sequencing of one bead by mass spectrometer. However, the large diversity of PEG derived signals were encountered and peptide peaks could not be identified. The present library has been constructed by the "one peptide on one bead (OPOB) basis" applied by the split and combine method on TentaGel® S NH_2 (90 μm), which is a graft co-polymer of polystyrene and polyethylene glycol. The library contains ca. 200 million octa-peptide beads constructed from 19 natural and 5 non-proteinogenic amino acids which include two D-cysteines that were used for enzymatic resistance during bioassays. Completion of acylation was monitored by the Kaiser test and double coupling was employed for incomplete acylation. Intra-molecular disulfides were formed by on-resin cyclization in 10% DMSO (pH6.0) at room temperature for 4 days. Since one bead carries ca 100 pmole peptides (calculated from resin substitution), complicated sample pretreatment is not practical due to loss of sample. To overcome these restrictions and problems we have attempted to develop partial hydrolysis followed by characterization using LCMS. In the present study the peptide-bead was screened using an assay with human cancer cells. The whole procedure has been carried out using microscopic observation to find particular beads which attached cells. The peptide on the bead was liberated by partial acid hydrolysis under optimized conditions. The partial hydrolysate, which contained not only target peptide but also PEG-derived materials, was subjected to nano-scale LCMS to give the primary structure of the target peptide. Based on the MS analyses the target peptides have been individually synthesized and confirmed the target sequence using the same assay. In the present study we have screened 24 beads carrying individual peptides whose structures have been elucidated.

PWe-127

11:10 – 12:20

MsXelerator: A Software Platform for (Reactive) Drug Metabolite Detection and Identification using High-Resolution Mass Spectrometry and Post-Acquisition Data Mining

Marco Ruijken

MsMetrix, Maarssen, the Netherlands

Keywords:
Reactive Drug Metabolites, Identification, DataMining

Novel aspects:
A novel software platform combining multiple data mining algorithms has been developed for the detection and identification of drug metabolites.

Abstract:
Introduction :
Recent post-acquisition data mining tools applied to the detection of drug metabolites in complex matrices using high resolution mass spectrometry include : Mass Defect Filtering, Precursor Ion Filtering, Neutral Loss Filtering, Background Subtraction and High Resolution Isotope Pattern Filtering. Starting point for any of the above data mining tools can be either a targeted or non-targeted approach. Most of the above techniques have distinct advantages and disadvantages. Here, we report on a new software platform combining the above mentioned algorithms and results into one automatic procedure. Combining the results from different filters significantly decreases the chance of missing important metabolites.

Methods :
After high resolution peak picking using the full scan MS data, the following algorithms are available : Mass Defect Filtering, Neutral loss and Product Ion Filtering, High Resolution Background Correction and Isotopic Pattern Matching for detection of metabolites containing Cl, Br or a mixture of a drug and its stable isotope labeled drug (reactive metabolite detection) . Filters can be used individually or sequentially in any selected order. In each step, peaks that conform to the applied filter can be deleted or marked. Most software tools will report a "filtered total ion current " after each step. The result of the MsXelerator software implementation is a table of ions that pass or fail each test. Identification is done by searching Metabolite Prediction lists.

Preliminary Results :
Several commercial test compounds were incubated with human liver microsomes for 60 min. Some other test compounds, Nefazodone, Verapamil, Diclofenac, Imipramine, Ticlopidine and Clozapine were incubated in rat liver microsomes with a mixture of GSH/stable isotope labeled GSH at a 1 : 1 ratio for 30 minutes or using KCN/$K^{13}C^{15}N$.
In order to assess the formation and detection of (reactive) metabolites in a discovery stage, and to test the MsXelerator performance, several ultra-high resolution mass spectrometer platforms were used and compared : the LTQ-Orbitrap and Velos and the Sciex Triple TOF 5600.
Incubation samples were injected and full-scan MS and MS/MS datasets were recorded. For stable isotope labeled samples, data dependent MS/MS was performed on the top two most intense peaks that have a mass difference of 3 Da (both high and low masses of mass tag) with a relative ratio of 80-100%.

Isotope Pattern filtering in combination with the post-processing algorithms almost doubled the number of detected GSH adducts compared to more traditional detection methods that are normally applied in this application field. In all cases IPF outperformed direct Neutral Loss analysis, especially for low abundant metabolites. Probably the most important step is the removal of common peaks, present in both sample and control by using the background correction algorithm. It is preferred to use this filter as a first step in the analysis. In general it can be concluded that product ion and neutral loss filtering are very useful as a confirmatory information source. It is not advised to apply a "hard filter " on these tools. The same is true for mass defect filtering. In many cases it was observed that mass defect filtering removes some of the unexpected metabolites.
A typical analysis, combining all post acquisition tools takes about one 1 - 2 minutes.

PWe-128

13:30 – 14:40

Non-targeted identification of novel Buspirone metabolites using a Spectral Similarity score derived from common fragment ion and neutral loss species

Kirsten Hobby, Alan Barnes, Neil Loftus

Shimadzu MSO, Manchester, UK

Keywords:
drug metabolism IT-TOF buspirone

Novel aspects:
Identification of novel pyrimidine ring cleavage metabolites of Buspirone via spectral similarity correlation score

Abstract:
Buspirone is an anxiolytic psychoactive drug of the azapirone chemical class, and is primarily used to treat generalized anxiety disorder (GAD) . Buspirone is a common model compound for xenobiotic metabolism. Here we describe the application of a Spectral Similarity Correlation methodology ('S Score') to the analysis of data generated from a human microsomal incubation of Buspirone. Non-targeted data-dependent MSMS data was collected from a human microsomal incubate using a Shimadzu LC IT-TOF system. The 'S scores' were derived from the correlation of fragment ion and neutral loss species of unknown chromatographic peaks to those of Buspirone. This approach was unbiased and success was not influenced any user interaction.

Buspirone (30µM) was incubated with human liver microsomes and NADPH at 37° C for 45 minutes. 5 µl of incubate was injected using a Shimadzu LC IT-TOF system onto a 2.1 x 50 mm C18 BEH 1.7um ; A - Water + 0.1 % formic acid ; B - Acetonitrile + 0.1 % formic acid. Flow 0.6ml/min @ 65° C. Gradient : 2 %B 0 min, 40%B 8 min, 90%B 9 -10min, 2 %B 10.5min.

The Spectral Similarity scoring was performed by Shimadzu Met ID solutions software (v1.2) . Two data files were used in the analysis. The t 0 sample was used to supply reference fragment ion and neutral loss data for Buspirone, and a t45 sample was treated as the unknown.

Initial data analysis by Met ID Solutions software was performed without a search for 'expected' metabolites and 37 peaks were returned with an S Score >0. Re-analysis by the software with a list of expected metabolites containing all previously described Phase 1 metabolites reduced the number of candidate 'unknown' peaks to 12. Manual scrutiny of the data indicated that some of the unknown peaks could be attributed to combinations of dealkylation and oxidative metabolic reactions but two peaks (m/z 350.2531 & m/z 438.2707) could not be assigned to previously described metabolites of Buspirone. Further analysis of the MSMS data supported the hypothesis that m/z 350.2531 was a product of cleavage of the pyrimidine ring in Buspirone. The m/z 438.2707 ion was tentatively identified as an intermediate in the metabolic cleavage of the pyrimidine ring as MSMS data showed the m/z 350 ion as a fragment.

Analysis of the literature showed that pyrimidine ring cleavage has been previously described for a structurally similar drug. The metabolic cleavage of the pyrimidine ring results in a loss of C_3 relative to Buspirone. In the absence of prior knowledge of this metabolic step it is unlikely that a metabolism scientist would search for metabolites containing the C_3 mass difference.

PWe-129
11:10 – 12:20

Analysis of interaction between TNF-alpha and anti-TNF-alpha agents by hydrogen deuterium exchange/mass spectrometry

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Keywords:
TNF-alpha, HDX/MS, anti-TNF-alpha

Novel aspects:
We found that a common region in TNF-alpha is shielded from the solvent when it is bound to four of anti-TNF-alpha therapeutic agents

Abstract:
Anti-TNF-alpha agents are used in treatment for some autoimmune diseases or inflammatory diseases like rheumatoid arthritis. There are several biological anti-TNF-alpha agents approved, one is a decoy receptor, and others are monoclonal antibodies. Although they are directed against identical antigen, they were established independently, thus their complementarity-determining regions (or ligand-binding regions) differ from each other. Then, what kind of structural differences are there when different anti-TNF-alpha agents capture TNF-alpha? For the purpose of elucidating this, we applied hydrogen deuterium exchange/mass spectrometry (HDX/MS) to TNF-alpha in the presence and absence of anti-TNF-alpha agents. HDX/MS is a known method which has its advantage in analyzing the dynamic aspects of protein higher order structures.
We examined four anti-TNF-alpha agents ; infliximab, a chimeric antibody, adalimumab and golimumab, human antibodies, and etanercept, a fusion protein of TNF receptor and IgG Fc domain. We immobilized anti-TNF-alpha agents on beads, deuterated TNF-alpha trimers on the anti-TNF-alpha beads or without anti-TNF-alpha agents, then quenched and eluted TNF-alpha with acid, and determined the regions which have differences in deuteration levels between in the presence and absence of the anti-TNF-alpha agents. Interestingly, the same region of TNF-alpha had exhibited decreased deuteration levels when TNFalpha was bound to anti-TNF-alpha agents, whichever anti-TNF-alpha agents it was bound to. The region we identified is supposed to be tucked between two TNF-alpha monomers in the trimer, and it also disagrees with the reported epitopes. These results and facts suggest that it possibly is a region which has been shielded from the solvent by the conformational changes induced by anti-TNF-alpha agents.

PWe-130

13:30 – 14:40

Validation and application of UHPLC-ESI-MS/MS method for the quantification of probe-drugs and metabolites of CYP3A4 and UGT1A1 in pharmacokinetics study

Daryl Kim Hor Hee, Zhangyan Yao, Lawrence Soon-U Lee

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Keywords:

Pharmacokinetics, probe drugs, metabolites, CYP 3 A4, UGT 1 A 1

Novel aspects:

Simultaneous quantification of probe-drugs, metabolites, inhibitors and inducer of metabolic enzymes CYP 3 A 4 and UGT 1 A 1 and its application in clinical trial pharmacokinetics study

Abstract:

An UHPLC-ESI-MS/MS based method for the quantification of metabolic enzymes CYP 3 A 4 and UGT 1 A 1 probe-drugs (midazolam and raltegravir, respectively) and metabolites (1'-hydroxy midazolam, 1'-hydroxy midazolam glucuronide and raltegravir glucuronide) simultaneously with the inhibitors (ketoconazole and ritonavir) and inducer (rifampicin) was developed. Analytes were extracted from 100 µl of plasma using Captiva™ ND^{Lipids}. Chromatographic separation was performed on a reverse phase C18 column (50 mm × 2.1 mm × particle size 1.8 µm) with a stepwise gradient using water and acetonitrile containing 0.1 % formic acid, at a flow rate of 0.5 - 0.6 ml/min. Separation of the 8 analytes was completed within the analytical run time of 4 min. The triple quadrupole mass spectrometer was operated in the positive ionization mode and drug quantification was performed using multiple reaction monitoring with deuterated internal standards. The method has been validated according to the FDA guidelines. The assay was linear over the concentration ranges that covered various analytes in the plasma samples. The lower limit of quantification for midazolam, 1'-hydroxy midazolam, 1'-hydroxy midazolam glucuronide, raltegravir, raltegravir glucuronide, ketoconazole, ritonavir and rifampicin were 0.1, 0.05, 0.2, 1, 1, 50, 50 and 50 ng/ml, respectively, in plasma. The method was proven to be specific, sensitive and robust. Accuracies and precisions of intra- and inter-assay at all concentration levels for various analytes ranged from 88.1 to 111.7 % and 0.4 to 13 %, respectively. The validated method has been successfully applied to the clinical trial pharmacokinetics study of inter-ethnic differences in the degree of induction and inhibition of CYP 3 A 4 and UGT 1 A 1 in a multi-ethnic Asian population and the results will be discussed.

PWe-131
11:10 – 12:20

MALDI High Resolution Mass Spectrometry in the Identification of Atorvastatin and Its Oxidative Metabolites

Robert Jirasko[1], Veronika Jedlickova[1], Tomas Mikysek[1], Michal Holcapek[1], Ales Svatos[2], Martin Kunes[3], Milan Nobilis[3]

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Keywords:
drug metabolites, Orbitrap, MALDI imaging, UHPLC/MS, electrochemical generation

Novel aspects:
Electrochemical generation of atorvastatin oxidative products and choice of suitable MALDI matrix followed by MALDI imaging high resolution mass spectrometry of atorvastatin in rat tissues.

Abstract:
The choice of suitable analytical techniques for the sample characterization in the area of drug metabolite analysis is an important task. Common analytical tools - combination of separation techniques and mass spectrometry (MS) are usually used for the analysis of body fluids, but this technique is unable to localize the distribution of particular compounds in the studied tissues and organs. The most widespread MS imaging scanning method is matrix-assisted laser desorption/ionization (MALDI) with a prominent role in the analysis of synthetic polymers and biopolymers. Nowadays, the MALDI is also considered in the area of small molecule analysis. The successful application of this technique is, however, hampered by low molecular weight matrix-derived interference signals and poor reproducibility of signal intensity arising mainly from inhomogenity of matrix deposition. The sublimation of suitable matrix followed by MALDI high resolution mass spectrometry can be used to avoid these complications.
In our study, various matrices typical for the small molecule analysis, such as 2,5-dihydroxybenzoic acid (DHB), its sodium salt (NaDHB), 2,4,6-trihydroxyacetophenone (THA) and 9-aminoacridine are tested on the mixture of atorvastatin and its electrochemically generated oxidative products previously identified by UHPLC/MS/MS. Experiments are performed using high resolution MALDI mass spectrometer LTQ Orbitrap XL equipped with nitrogen UV laser (337 nm, 60Hz). Tandem mass spectra are also measured to obtain supplementary structural information. The most suitable matrix with regard to atorvastatin signal enhancement is further studied in the atorvastatin distribution in rat tissues. The matrix coating is done by the sublimation to obtain small crystal size and avoid local variations in the ionization efficiency.

The financial support of this project was provided by the Czech Science Foundation (No. P206/12/P065).

PWe-132 Metabolic Stability Screening Workflow using a Second Generation High Resolution Accurate Mass Benchtop Instrument

13:30 – 14:40

Shigeru Sakamoto[1], Sun Hai-Ling[3], Soohoo Daniel[3], Comstock Kate[2], Stratton Tim[2]

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Keywords:
Benchtop-Orbitrap MetQuest Quan/Qual metabolic-stability-screening

Novel aspects:
Ultra high resolution mass spectroscopy, automated software acquisition and processing for simultaneous relative quantitative and qualitative (Quan/Qual) analysis.

Abstract:

Introduction
In vitro metabolic stability screening performs a very important role in the drug discovery stage for compound selection in pharmaceutical companies. The screening is primarily supported by LCMS, which involves the monitoring of the disappearance of parent compounds, using selected reaction monitoring (SRM) on triple-quadrupole instruments. If moderate to high turnover is observed, separate metabolite identification experiments are then conducted to characterize the biotransformation products. In this study, we present a novel workflow using a high resolution accurate-mass benchtop Mass spectrometer, Exactive. This workflow combines relative metabolic stability and initial metabolite information from the same analysis. The high mass resolution with high scan speed data acquisition is compatible with UHPLC for high throughput screening.

Methods
Six model compounds were selected for this study. Compounds (3 µM) were incubated using human, dog and rat hepatic microsomes at nominal 0.5 mg protein/mL at 37° C for up to 60 minutes in the presence of NADPH. At 0, 5, 15, 30, 45 and 60 minutes, aliquots of the reaction mixture were transferred and mixed with quench solution. The samples were centrifuged, and supernatant was injected for analysis. The time-concentration profiles of parent compounds and identified metabolites were estimated from the acquired data by using an Exactive II benchtop HR mass spectrometer in positive full scan / all ion fragmentation (AIF) scans at 70,000 FWHM resolution. Simultaneous acquisition and processing were performed using the relative Quan/Qual software package, MetQuest 1.1.

Preliminary Data
The metabolic stabilities of six diverse compounds- trifluoperazine, carbenoxolone, raloxifene, diclofenac, paclitaxel, and piroxicam were analyzed by UPLCMS on a benchtop Orbitrap MS with higher energy collision dissociation (HCD) . Data were acquired as a full scan followed by an all-ion-fragmentation (AIF) scan. The data acquisition was conducted by using MetQuest 1.1 metabolic screening software, which performed automatic relative Quan/Qual data analysis. Further metabolite structure determination was possible using the same data files, without reinjection, by using data mining software. Initial processing of the time-course samples was performed automatically, using a list of 50 mass changes representing commonly found biotransformations as well as screening for unexpected metabolites. To be considered an unexpected metabolite, the extracted ion chromatogram of an observed m/z value had to follow a peak shape, and not appear in the control sample. Expected metabolites were included only if the area, after comparison to the control, differed by more than 5 X and whose exact mass value was within 5 ppm of the expected value. This workflow reduces data turnaround time, increases compound throughput, and maximizes mass spectrometer usage.

PWe-133
11:10 – 12:20

Development of High Performance Liquid Chromatography Tandem Mass Spectrometry Method for Analysis of Bacopaside-I in Rat Urine and Feces Samples

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Keywords:
Pharmacokinetics, Quantification, LC-MS/MS, Validation

Novel aspects:
A LC-MS/MS quantitative analysis method of Bacopaside I in rat urine and feces samples method has been developed and validated

Abstract:
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Introduction

Bacopaside I ($C_{46}H_{74}O_{20}S$) is one of active components isolated from *Bacopa monnieri (L.)* Wettst. (Brahmi) , which has been used as an Ayurvedic medicine plant for centuries [1] . It has been reported that Brahmi has potential therapeutic effect in treatment and prevention of neurological diseases and improvement of cognitive processes [2,3] . Memory enhancing effect of Brahmi has been established through animal experiments and in healthy volunteers. The damarane triterpenoid saponin, Bacopaside I is a major active compounds in Brahmi. HPLC method [4] has been established and used to determine the bacopaside I and other active components in the plant extracts as well as biological samples [3] . However, so far LC/MS method for quantitative analysis of Bacopaside I in biological samples has not been reported. We report for the first time the development and validation of a LC-MS/MS method, aiming for pharmacokinetic study of bacopasides I.

Experimental

A LCMS-8030 (Shimadzu Corporation, Japan) tandem triple quadrupole LC-MS/MS was used for this method development study. A fast gradient elution separation program on a Kinetex C18 HUPLC column (1.7 um, 50 mmL x 2.1mmID) was developed and optimized. The MRM transitions in ESI positive mode used are 979.4 > 473.4 for bacopaside I and 609.3 > 195.0 for reserpine as internal standard. Rat urine and feces were used as the biological matrix for method development and validation of bacopaside I quantitative analysis. Liquid-liquid extraction method was employed in sample extraction and purification.

Results and Discussion

The performance of the method has been evaluated systemically using extract samples prepared from rat urine and feces. The linearity (r^2) was 0.9999 for a range from 8.6 ng/mL to 900 ng/mL. The LOQ of the method was lower than 8.6 ng/mL. The peak area reproducibility at this concentration level (spiked into urine) was 17.2% (RSD, n=6) . The peak area reproducibility were 6.3& and 2.9% for concentration levels of 25.7 ng/mL and 85.5 ng/mL, respectively. The recoveries of bacopaside I spiked into urine were between 100% and 137.1% for three concentration levels (50, 80 and 100 ng/mL) . However, the recovery of feces extract samples was found lower than that of urine samples at 30~60%. Further study to improve the recovery of bacopaside I in feces samples is undergoing. The method has been applied to *in vitro* hydrolysis samples.

Conclusions

For the first time, a high sensitivity MRM method on LC-MS/MS was developed and validated using rat urine and feces samples. The method with further improvement in feces sample pretreatment to improve the recovery is ready for use in pharmacokinetics study.

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PWe-134

13:30 – 14:40

Novel Mass Spectrometry Based Method for Quantitation of Antibody Drug Conjugates

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Keywords:
ADC stability quantitation affinity cytotoxin

Novel aspects:
Novel method for quantitation of ADCs based on affinity capture of ADC, release of the ADC bound drug by digestion and quantitation of the released free drug by mass spectrometry.

Abstract:
Potency and specificity are desirable attributes of a successful drug. These qualities are implemented by antibody drug conjugate (ADC) constructs, where potent cytotoxic agents (drugs) are linked to antibodies with high specificity for the surface of targeted cells. The stability of the ADC as an intact entity is therefore of particular importance.
ADCs are typically quantified by ELISA after development of antibodies against either the antibody or the attached drug. However, in neither case is it assured that the ADC measured by ELISA is intact.
We therefore developed a mass spectrometry based method for quantitation of ADCs based on affinity capture of the ADC, followed by thorough washing to remove non-specifically bound material including free drug, and subsequent release of the ADC bound drug by digestion. The resulting free drug was quantified by mass spectrometry.
This work flow differs from a recently developed workflow by Xu et al 2011 in several respects. The here presented affinity capture does not require access to high purity antigen, and the quantitation method measures the drug released and extracted from the ADC directly by mass spectrometry, as opposed to deglycosylation and reduction of the IgG with subsequent mass spectrometric determination of the drug antibody ratio (DAR) values of the light and heavy chains.
Data will be shown that demonstrate the linearity of the approach and application hereof for ADC stability studies.
It should be underlined that in addition to measuring the ADC bound drug directly, this approach will be of particular value for comparative developments of novel ADCs, where different antibodies are linked to an array of drugs by different cleavable drug linkers, as development of individual ELISA kits for each ADC would be time consuming and expensive. The here presented workflow can seamlessly be altered for quantitation of any new cleavable linker based ADC.

Reference
Xu et al 2011, Anal. Biochem. 412 56-66

PWe-135

11:10 – 12:20

Method for the direct injection and on-line sample clean-up of whole methanolic extracts from dried blood spots

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Keywords:
DBS, On-line sample clean-up, At-column dilution, 2 D LC/MS/MS, Bioanalysis

Novel aspects:
Ability to inject whole organic DBS methanolic extracts with on-line sample clean up in a single injection.

Abstract:
Introduction

The collection of blood onto Guthrie-type filter paper has recently been demonstrated and is referred to as dried blood spots (DBS) . This sample preparation method has been demonstrated to reduce both cost and animal usage. Most often the analyte of interest is extracted from the DBS with neat methanol and must be diluted with water prior to LC/MS/MS analysis. This dilution step therefore influences assay sensitivity. In this work, we present the use of 2 D LC/MS/MS coupled with at-column dilution (ACD) for the analysis of rosuvastatin from DBS extracted with neat methanol. This approach enabled maximum sampling of the whole extract onto the 2 D LC/MS/MS system and on-line sample clean up in a single injection.

Methods

An 80 µL aliquot of the methanolic DBS extract was injected onto the 2 D LC/MS/MS system. ACD was performed using a 50 µL mixer prior to the trapping column. Following sample loading onto the trap column for 0.5 minutes, the trap column was washed with 20 percent acetonitrile for 0.5 min. The valve was then switched and the sample forward flushed onto the analytical column. Rosuvastatin was eluted from the analytical column at 500 µL/min under a linear gradient from 5 -55 percent acetonitrile in 5 min. Mass spectrometry was performed on a Xevo TQS operating in positive elctrospray ionization mode and in MRM acquisition mode.

Preliminary Data

An assay was developed by utilizing a heart-cut configuration and at-column dilution for the methanolic extract of rosuvastatin DBS. The at-column dilution of the rosuvastatin DBS allowed for more sample to be loaded onto the system, producing greater peak height and improved peak shape, thus producing a low LLOQ for the assay. The assay proved linear for 2.5 orders of magnitude with all residual values less than 9 %, producing an assay within bioanalytical validation criteria as defined by the FDA. The LLOQ of the assay was determined to be 0.1 ng/mL with a signal to noise value of greater than 5 : 1. Carryover was evaluated by injection of a matrix blank following the ULOQ of 50 ng/mL. We observed no detected analyte in the matrix blank. Interferences both from the DBS card as well as the matrix where reduced via the heart cutting method as well as contamination of the MS source. Based on the results of this study, this method shows promise for other bioanalytical assays whereby sample (s) consisting of a high organic component can be directly injected and on-line clean up performed in a single injection.

PWe-136
13:30 – 14:40

Metabolite analysis using a novel relationship and database driven software platform approach for screening and understanding metabolism

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Keywords:
Metabolite identification, Database, Network, UPLC, QTof

Novel aspects:
A networked platform integrating advances in multi-parametric use of data to identify drug related material and disseminate to global organisations

Abstract:
Introduction

Informatics is increasingly becoming the key component in driving knowledge generation from DMPK LC/MS studies. Current software platforms are generally limited to single experiment analysis types and although these approaches continue to serve us well, the majority of these platforms were designed for linear experimental designs. As analytical methods deployed in DMPK have delivered improvements in data quality and throughput , researchers are focusing on ways to increase efficiency by deriving greater value from their complex datasets. The approach defined is a software system that uses the latest computing hardware (64-bit, multi-core capabilities) , latest generation hardware (UPLC/MS Qtof platforms) and software (scientific library data management) to accurately screen, confidently interrogate datasets and report the data in a highly customizable format.

Methods

Metabolite analysis for several compounds was performed using a prototype acquisition and processing software. Central to this informatics platform is a scientific library which stores information related to compounds of interest providing a starting point for developing methods as well as capturing the results of the analysis. From here samples were automatically processed to provide a comprehensive list of metabolites which can then be interrogated to suit both the analytical and reporting needs of the user. We will discuss the use of data properties such as ; mass accuracy, decimal mass analysis, isotopic pattern and fragment correlation analysis to drive a filtering process that enables unbiased identification of drug related components and allows the user to establish and map metabolite relationships.

Preliminary Data

Data set was processed and directly compared with the current generation of metabolite screening software. Data was processed and analyzed using newly integrated Apex peak picking algorithms, taking advantage of the multicore processing capabilities of modern GPU based technology which has shown an improvement of >3 fold in the time required for extraction and processing raw data. Additionally, processing is centralized around the built in scientific library functionality which manages both compound and metabolite identification specific criteria. This advanced decision making results in a transparent user experience which automatically adapts to the unique chemical properties of a compound allowing users to generate metabolite relationships within a single LCMS software package as opposed to current methodology relies heavily on linear single compound, single dataset, single answer approaches. With a scientific library underpinning, we are able to both build and retrieve metabolite relationship information across datasets and samples that were historically difficult to manage as a single report. Stronger integration of in silico tools, metabolite prediction, isotopic pattern analysis and more flexible mass defect filtering have been used in order to more confidently assign drug related materials. In this presentation we will demonstrate how drug metabolism departments will have the ability to manage instrument and server assets remotely across the network, this network based infrastructure also facilities scientifically sharing and disseminating data and knowledge across labs and across networks. DMPK datasets have been processed for both generating qualitative structural and relationship pathways as well as for quantitative analysis and putting the datasets into meaningful context within a single, integrated processing and reporting environment.

PWe-137
11:10 – 12:20

HILIC-UHPLC-MS/MS determination of entecavir in rat plasma, urine and plasma ultrafiltrate using various sample preparation techniques

Lucie Novakova[1], Hana Vlckova[1], Tomas Gottvald[1], Jaroslav Janak[1], Frantisek Trejtnar[2], Jana Mandikova[2], Petr Solich[1]

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Keywords:
entecavir, UHPLC, HILIC, MS/MS, biological material

Novel aspects:
A new HILIC UHPLC-MS/MS method for the determination of entecavir in various biological material was developed and validated. Several approaches were used to reduced matrix effects.

Abstract:
Entecavir is a novel deoxyguanosine nucleotide antiviral agent with activity against hepatitis B virus (HBV). The agent possesses a polar structure, which might cause difficulties, when determined by conventional LC-MS methods. In this study RP-UHPLC method using BEH C18 stationary phase with mostly aqueous binary mobile phase composed of (4 : 96) acetonitrile/0.01% formic acid and HILIC-UHPLC method using BEH Amide stationary phase with binary mobile phase composed of acetonitrile/5 mM ammonium acetate pH 4.0 (75 : 25) at flow-rate 0.3 ml/min were compared in terms of sensitivity, linearity, repeatability and accuracy. The effect of various mobile phase additives (formic acid, acetic acid, ammonium acetate and ammonium formate at both acidic and basic pH) was carefully studied in both RP and HILIC method.
HILIC-UHPLC-MS/MS method demonstrated more favorable results and moreover, it provided more convenient conditions for straightforward coupling with solid phase extraction-based (SPE) sample preparation techniques. Several biological materials (rat urine, rat plasma and plasma ultrafiltrate of rats) were examined using various SPE-based extraction techniques (RP-SPE, ion-exchange SPE and microextraction by packed sorbent (MEPS) approach).
The sensitivity of developed UHPLC-MS/MS method was relatively higher compared to previously published works. Method validation in terms of linearity, accuracy, precision, selectivity and matrix effects was performed for each of biological material. By the combination of several approaches, that enable to reduce matrix effects (relatively selective SPE sample preparation step, efficient UHPLC separation, HILIC mode enabling different selectivity and high sensitivity, quantitation using selective SRM mode and SIL-IS with $C^{13}N^{15}$ labeling and finally sample dilution), resulting method provided accurate results with negligible matrix effects and still high sensitivity sufficient for the determination of entecavir in rat urine, plasma and plasma ultrafiltrate samples in spite of the dilution step during sample preparation.

The authors gratefully acknowledge the financial support of research projects of Charles University in Prague UNCE 17/2012 and PRVOUK.

PWe-138
13:30 – 14:40

Determining The Pharmacokinetics And Metabolic Fate Of A Novel Anti-Cancer Compound Using LC/MS/MS With Comprehensive Data Collection

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Keywords:
qualitative and quantitative analysis, Tandem quadrupole MS, Q-Tof, Bioanalsyis, Metabolite identification

Novel aspects:
Quantification over a wide dynamic range using a hybrid QToF instrument with simultaneous acquisition of qualitative data.

Abstract:
Introduction

The aim of preclinical drug metabolism is to determine the safety profile of a drug candidate. As part of the process it is necessary to quantify the compound or active moiety and provide information on the metabolic fate of the molecule. In this paper we present a comparison of the benefits and challenges for the simultaneous acquisition of qualitative and quantitative LC/MS/MS data using either a QToF or tandem quadrupole instrumentation. The throughput, sensitivity and spectral quality were compared using plasma samples derived from oral administration of a novel oncology compound to the rat and dog. The samples were deprotinated and analysed by accurate mass LC/MS and tandem quadrupole LC/MS/MS.

Methods

Plasma was collected following the oral administration of a novel oncology compound over four weeks and processed via protein removal with a phospholipid preparation plate. The chromatography was performed on a 2.1 x 50 mm 1.7 μm C18 column was maintained at 50 ° C and eluted with a basic aqueous methanol 5 minutes gradient at 600 μL/min. The column eluent was monitored by positive electrospray TOF MS, across mass range 50 1200 m/z and on a tandem quadrupole MS using MRM and data dependent MS. The compound was quantified using a stable-label isotope and authentic standard. The metabolites were detected and identified by either accurate mass and comparison of the fragment ion spectra or via data directed MS/MS on the tandem quadrupole.

Preliminary Data

The tandem quadrupole mass spectrometer was used to simultaneously acquire LC/MS data in two modes ; i) MRM to acquire quantitative data for the analytes and ii) data directd MS/MS to obtain qualitative data for metabolite detection. The QToF was operated in alternating low and elevated energy mode allowing the simultaneous collection of both precursor and product ion data. The QTof was operated with a resolution of 20,000 FWHM and a data collection rate of 20 spectra-per-second. This resolution allowed the use of a narrow mass window for data processing ; in this case it was possible to employ a 0.5mDa window. The data collected from both instruments showed the presence of four major metabolites ; two hydroxylated metabolites, a demethylated metabolite and a dealkylated metabolite. The Cmax for the metabolites occurred at 3 hours with all the metabolites being cleared by 24hrs. The high degree of selectivity of the QTof produced excellent signal/noise resulting in an LOD of 50pg/mL linear over 4 orders of magnitude. The metabolites were detected by the use of the accurate mass precursor and the structure elucidated using the accurate mass fragment ions produced in the high collision energy data. The limit of detection for the tandem quadrupole mass spectrometer in MRM mode was determined to be 10pg/mL. Whilst the triple quadrupole mass spectrometer showed greater sensitivity for quantification, the full scan data used for metabolite detection and identification was approximately 50-fold less sensitive than the QToF. The specificity of the tandem quadrupole instrument for metabolite detection was increased by using a data directed approach ; here the MRM transition for a series of potential metabolites using knowledge of the parent fragmentation pattern and potential biotransformations. When a potentially metabolite related peak was detected the instrument automatically collected MS/MS data during the trailing edge of the chromatographic peak.

PWe-139

11:10 – 12:20

Development of a Highly Sensitive Methodology for Quantitative Determination of Fexofenadine in Microdose Studies by Multiple Injection Method Using UHPLC-MS/MS

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Keywords:
multiple injection method, ultra-performance LC-MS/MS, fexofenadine, microdose study, high sensitivity

Novel aspects:
This study suggests that HSMIM has an ultra high sensitivity and high-throughput for determining by LC-MS/MS using a simple sample preparation in PK and microdose studies.

Abstract:

Introduction

Recently, the trend has been toward lower efficacy doses because of the synthesis of many compounds with strong activity, quantitative methods utilizing LC-MS/MS with higher sensitivity are required. LC-MS/MS with high sensitivity and selectivity is the most informative method with respect to structural analysis of drug metabolites and determination of drug concentrations in biological samples. Fexofenadine, a non-radiolabeled compound, is an antihistamine drug that was orally administrated at a dose of 100 μg/man/day in the first microdose clinical study in Japan in 2005 ; subsequently, the concentration of fexofenadine in human plasma was measured by LC-MS/MS. The lower limit of quantification (LLOQ) was 10 pg/ml when the concentration of fexofenadine in human plasma, prepared by solid-phase extraction, was determined using LC-MS/MS. Previous studies have frequently used the protein precipitation method using an organic solvent for sample treatment and the LLOQs in the "ng/ml " range to determine the concentration of fexofenadine in biological samples by LC-MS/MS. However, the drug concentration of samples in a microdose study cannot be measured at an LLOQ of only ng/ml because of lack of sensitivity. In case of employing solid-phase extraction or liquid-liquid extraction for sample preparation, it is possible to set the LLOQ at pg/ml, but total sample analysis is required, which demands increased time as the procedure of sample preparation is quite complex. In pharmacokinetic (PK) studies with many samples, solid-phase extraction method is disadvantageous, as it is more expensive than the protein precipitation method. In this work, fexofenadine was selected as a model compound, and we developed and validated the novel method, ultra high-sensitivity quantitative method of multiple injection method (HSMIM) , for both rat and human plasma samples without an internal standard compound by using simple sample treatment procedures like the protein precipitation method. Furthermore, we demonstrated the analytical time required by using the ultra-HPLC (UHPLC) technique.

Methods

The UHPLC system used was Nexera (Shimadzu Corporation, Kyoto, Japan) . The mass spectrometry-based detection was performed on an API 5000 triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA, USA) . The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode using positive-ion electrospray ionization. HSMIM consists of 2 steps. Step 1 involves injection and condensation : the same compound is continuously injected into a column and trapped at the column head under an isocratic condition of low organic solvent concentration. Step 2 involves elution : the compound trapped at the column head is eluted and introduced into the mass spectrometer.

Results

The calibration curves of fexofenadine prepared using rat plasma and determined by HSMIM were fairly linear over the concentration range of 5-5000 pg/mL. The intra-day precisions of fexofenadine were <8.7%, and the accuracies ranged from 95.2% to 99.3%. The inter-day precisions were <8.4%, and the accuracies ranged from 98.6% to 102.6%, respectively. The concentrating effect of fexofenadine on the column was investigated by HSMIM by using rat plasma samples. Consequently, we confirmed that the peak area of fexofenadine in HSMIM showed an approximately 5-fold increase compared to the conventional method. In addition, reduction of the analytical time and the sharper peak were achieved by applying the UHPLC technique to HSMIM. We were able to set up a high-throughput analytical method in which the total analytical time was approximately only 2 min per sample, and the retention time of fexofenadine was about 0.6 min. The concentration in rat plasma after oral administration of 100 μg/kg fexofenadine (1/100th of the efficacy dose in rat) was determined by this validated method. As a result, the plasma concentrations of all samples could be adequately measured using this method.

PWe-140
13:30 – 14:40

Mass spectrometric analysis of ginsenosides in Panax ginseng root and callus using pseudo two-dimensional liquid chromatogram

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Keywords:
pseudo 2D chromatogram, LC-MS, Orbitrap, ginsenosides, Panax ginseng

Novel aspects:
A rapid and simple method for creating a pseudo 2D chromatogram using conventional LC-MS was developed and applied to analysis of ginsenosides in the calli and root of Panax ginseng.

Abstract:
Introduction
Panax ginseng C.A. Meyer is one of the most famous herbal products available and the root of Panax ginseng is used in some traditional Chinese medicine treatments. The root contains many bioactive compounds of which its major class is glycosides, which includes ginsenosides. It is important to understand what effective combinations and appropriate amounts of glycosides should be used in drug discovery and pharmacology. Recently, on-line two-dimensional (2D) liquid chromatography (LCxLC) methods that use two different columns with different chromatographic behaviors have been developed. But it takes longer times to determine the LC conditions and create the LCxLC chromatograms. Therefore we developed a rapid and simple method for creating a pseudo 2D chromatogram using conventional LC-MS for analysis of ginsenosides.

Methods
The samples were extracts of the calli and the root of Panax ginseng (Zen-Noh, Japan). Dried 500 mg samples were prepared by sonication in 70% aqueous methanol. The sample solution was centrifuged and the obtained solution was measured by LC-MS (Exactive, Thermofisher Scientific). LC-MS chromatography data of retention time (RT), m/z, and ion intensities were digitalized using the software program SIEVE (ver.1.2, Thermofisher Scientific). The noise level of the ion intensity was set to an appropriate value to remove insignificant peaks. The LC-MS chromatography data were extracted with each use of the reverse phase and HILIC columns. A scatter diagram of the retention times for the pseudo 2D chromatogram was created on the basis of the m/z values.

Results
The standard solution of seven ginsenosides was analyzed using LC-MS with the reverse phase or HILIC column. We found that ginsenosides Rg1 and Re were only separated by the HILIC column. In contrast, the Rg1 and Rf isomers were only separated by the reverse phase column. A pseudo 2D chromatogram was created from the two chromatograms to solve the problem of separation. The RT values for the HILIC and reverse phase chromatograms were plotted along the abscissa and the ordinate of the pseudo 2D chromatogram, respectively. The pseudo 2D chromatogram showed that the plots for Rg1 and Rf, which were eluted together by HILIC LC-MS, and the plots for Rg1 and Re, which were eluted together by reverse phase LC-MS, could be separated and visualized. The pharmacological properties of callus A and the root of Panax ginseng are different, possibly because of the different amounts and types of ginsenosides that they possess. Therefore, it is significant when analyzing their pharmacological properties to determine the components that are present in their extracts rapidly. Extracts derived from the calli and roots of Panax ginseng were analyzed by the pseudo 2D chromatogram. The differences in the components found when comparing the callus and root, or callus and another callus that were cultured under different culture conditions, were distinguished by crosschecking their pseudo 2D chromatograms against one another. We found that the components contained in the calli and their quantities were altered by the culture conditions in which the calli were grown. The pseudo 2D chromatogram is expected to be a useful method for visualizing complex component patterns found in glycosides and unknown compounds in foods.

PWe-141
11:10 – 12:20

Metabolic site elucidation of glucuronide metabolites using ion mobility and molecular modeling

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Keywords:
Ion mobility spectrometry, Metabolite identification, glucuronide metabolites, Positional isomers

Novel aspects:
Ion mobility technology delivers the new approach for structure elucidation of glucuronide metabolites based on comparison of collision cross-section of metabolites.

Abstract:
[Introduction]
The identification of drug metabolites is a critical component in drug discovery and development process. It supports the selection of species for the toxicology study, and provides information on the potential to form pharmacologically active metabolites or metabolites with toxicological consequence in human. Tandem mass spectrometry coupled with liquid chromatography (LC/MS/MS) has commonly been used as the first step of metabolite structure elucidation. However, collision-induced dissociation (CID) often fails to identify the site of biotransformation. Especially product ions of glucuronide metabolites are lacking in information about biotransformation site, because glucuronic acid moiety as a neutral fragment is easily loosed from precursor ion. In such case, metabolite isolation is needed and NMR is typically employed to obtain structure information, and this approach can be including time consuming process. Ion mobility spectrometry (IMS) has the ability to separate isomeric species such as glucuronide metabolites, rapidly (msec) based on difference in collision cross-section (W) in the gas-phase, thus providing specific information on ionic configuration, i.e., the position of the glucuronidation.

[Method]
Glucuronide metabolite isomers of raloxifene and ezetimibe were used as model compounds. Experiments were performed using a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight Synapt G2 HDMS instrument coupled with ultra performance liquid chromatography (UPLC-IMS-MS, Waters Corp., Milford, MA) . Ion mobility separation was performed at a pressure of 3.2 mbar nitrogen with a wave velocity of 650 m/sec and travelling wave amplitude of 40 V was used. Theoretical Ω values were calculated using DriftScorp (Waters Corp., Milford, MA) , and compared to the travelling wave derived Ω values. Ion mobility calibration was undertaken using eight singly charged polyalanine ions.

[Results and discussion]
Raloxifene-4'-gluclonide (Rxf-4-G) and raloxifene-6-glucuronide (Rxf-6-G) showed different UPLC chromatographic elution time. However, the MS spectra and breakdown curve of product ions showed all the identical patterns, therefore the identification of metabolic site was difficult for these metabolites. The W values of Rxf-4-G and Rxf-6-G were measured by UPLC-IMS-MS. Theoretically calculated W value for Rxf-4-G and Rxf-6-G were 176.6 $Å^2$ and 180.7 $Å^2$, respectively. Collision cross-section values derived from travelling wave ion mobility were in very close to the theoretically derived values, thus allowing accurate identification of the glucuronide metabolites, even those with a collision cross-section difference of less than 1 $Å^2$.
Further, phenolic glucuronide of ezetimibe (Eze-P-G) and benzylic glucuronide of ezetimibe (Eze-B-G) were also separated with UPLC. MS/MS analysis of these ezetimibe glucuronides gave characteristic structure information for each compound. Product ion spectrum of Eze-P-G included a peak corresponding to the loss of water molecule from benzyl hydroxyl group moiety. On the other hand, the product ions from Eze-B-G did not show the peak of water loss because glucuronic acid is adducted to the benzyl hydroxyl group. In such case, MS/MS analysis provides information for elucidating the structure of isomeric metabolites. The major product ion from both glucuronides of ezetimibe is m/z 392 corresponding to neutral loss of both water and glucuronic acid from precursor ion. The IMS driftgram of m/z 392 suggested the presence of geometric (cis/trans) isomers. The intensity ratio of cis- and trans-isomers were different between Eze-P-G and Eze-B-G. This may be originating from the difference of conformation for each glucuronide.
In conclusion, IMS technology was a powerful tool for structure identification of isomeric species, such as glucuronide metabolite, without additional analytical techniques and chemical synthesis. Additionally, IMS technology suggested a possibility for providing information of relationship between conformation of gas-phase molecule and fragmentation mechanisms.

PWe-142 Mimicking of the phase I metabolism of drugs by photocatalytic reactions

13:30 – 14:40

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University of Helsinki, Helsinki, Finland

Keywords:
Titanium dioxide, Photocatalysis, Oxidation, Phase I metabolism

Novel aspects:
Titanium dioxide photocatalytic reactions for mimicking phase I metabolism of drugs.

Abstract:
The metabolism of a drug candidate has to be investigated early in the preclinical phase of drug development in order to predict the potential risks. Drug metabolism is studied *in vitro* using hepatocytes, microsomes or recombinant enzymes and the experiments are extensive, laborious and expensive. Due to these drawbacks, methods such as electrochemistry (EC) , Fenton reaction, EC-Fenton reaction and metalloporphyrin systems have been studied as alternatives to *in vitro* metabolism studies.[1-3] The reaction products of these oxidation methods have been shown to resemble the products of oxidative drug metabolism reactions, which are the most important phase I metabolism pathways. Also another oxidative system, namely titanium dioxide (TiO_2) photocatalysis, has been recently shown to produce reaction products which correlate with metabolites produced *in vivo* and with human liver microsomes *in vitro*.[4] TiO_2 is able to catalyze both reductive and oxidative reactions when exposed to UV light having high enough energy to excite electrons from the valence band to the conduction band of TiO_2. The aim of our study is to further investigate the feasibility of a TiO_2 photocatalytic system for imitation of phase I oxidative metabolism, compare it to electrochemical oxidation and develop a simple generic photocatalytic method using TiO_2 particles.

Photocatalytic reactions were carried out in liquid phase using titanium dioxide Degussa P25 particles. The reaction mixture was magnetically stirred during exposure to UV light. Titanium dioxide particles were removed from the reaction mixture by centrifugation and the supernatant was collected for mass spectrometric analysis. The reaction products were analyzed using appropriate ionization technique, electrospray ionization or atmospheric pressure photoionization, according to the properties of the analyte. TiO_2 photocatalytic reaction products of the selected drug molecules were compared with *in vitro* and *in vivo* metabolites and with electrochemical oxidation products found in literature.

The photocatalytic method is simple and relatively rapid. The preliminary results suggest that TiO_2 photocatalytic reactions have potential for imitation of phase I metabolism. Photocatalytic reactions could be particularly useful for production of metabolites in larger scale than is possible using *in vitro* methods.

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PWe-143 Streamlining the Metabolite Identification Workflow Using High Resolution QTOF Data and Mass-MetaSite.

11:10 – 12:20

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Keywords:
Predictive metabolite identification.

Novel aspects:
Metabolite identification based on matching MS/MS data and analysis of the potential interactions of drug compounds in the cytochrome P450 cavity and their chemical reactivity towards oxidation

Abstract:

Introduction :
The availability of high resolution accurate mass QTOF data has revolutionized the metabolite identification field. Nowadays, an expert can generate an unprecedented amount of accurate data for each chromatographic peak in order to assign the chemical structure of metabolites. Therefore, sophisticated data processing software is needed to help transform this huge volume of data into information that can be shared in an efficient manner.
This presentation demonstrates the use of Mass-MetaSite integrated with MassHunter file format, to identify drug related material peaks in the chromatogram, assign chemical structures for each of the peaks found.

Results :
The Mass-MetaSite process consists of two steps. The first step identifies Drug Related Material using the chromatogram obtained from the QTOF MS and MSMS methodology in order to find chromatographic peaks that are related to the parent compound. It reports 2 types of peak : a) peaks that correspond to a mass shift from the parent corresponding to one or more metabolic reactions (phase I and II) that are set prior the calculation within the software and b) unknown metabolites, these are observed peaks but do not correspond to any of the list of known metabolic transformations.
In the second step the chemical structures giving rise to the peaks are assigned. In order to perform this operation, the software produces a set of theoretical fragments (structure and mass) for the parent and the metabolites and the masses for that fragment list compared with the actual fragment ions found in the MS and MSMS spectra. From this comparison the software is able to localize the region in the molecule where the metabolic reaction may take place. In the case that the fragment analysis cannot specify a single atom in the parent that produce the metabolic reaction, a markush representation of the metabolites compatible with the mass spectra data is shown to the user and in order to prioritize among the different potential structural solutions the MetaSite Site of Metabolism (SoM) algorithm[1,2] is applied. The SoM prediction is used to predict the regioselectivity of the metabolic reaction only for phase I metabolism and it is based on an analysis of the potential interactions of the compounds in the cytochrome P450 cavity and the chemical reactivity towards oxidation.

Discussion :
We present an integration of QTOF high resolution accurate mass measurements with software that processes the data in order to suggest peaks and structural assignment of each peak to the metabolite identification expert via the automatic Mass-MetaSite batch process and the reporting system based on the WebMetabase application. The computational tool performs a systematic analysis of the fragmentation by breaking bonds to elucidate the structure of the metabolite and report to the expert the suggested structural fragments. In addition, when the mass spectral data is not enough to localize a definitive structure of the metabolite, the MetaSite SoM prediction algorithm is applied to suggest a potential metabolite that is in agreement with the spectral data.
The WebMetabase tool collects the data from the automatic procedure in Mass-MetaSite, and presents the data to the expert for approval, keeping all the fragments in a database. WebMetabase also allows the analysis of experiments with multiple samples, for example performing a kinetic analysis enabling the possibility to keep both qualitative and quantitative data in a single system.

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PWe-144
13:30 – 14:40

The development of multistatin method for determination of statins, their metabolites and interconversion products in human serum

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Keywords:
statins, UHPLC-ESI-MS/MS, sample pretreatment, MEPS, SPE

Novel aspects:
A novel method for determination of 17 analytes (statins, their metabolites and interconversion products) was developed and validated. Two sample pretreatment techniques (MEPS, SPE) were compared.

Abstract:
Statins decrease the levels of total cholesterol, low-density lipoprotein cholesterol and plasma triglycerides due to inhibition of microsomal 3-hydroxy-3methylglutaryl-coenzym A (HMG-CoA) reductase. They are used for the treatment of hypercholesterolemia in clinical practice. Recently anticancer effect was discovered for selected statins.

The aim of this project was to develop and validate UHPLC-MS/MS method and convenient sample preparation procedure for simultaneous determination of statins (atorvastatin, rosuvastatin, simvastatin, lovastatin, pravastatin, fluvastatin and pitavastatin), their metabolites and interconversion products in human serum. As the sample preparation technique solid phase extraction and microextraction by packed sorbent were used and compared.

Suitable conditions for UHPLC/MS/MS determination of 17 analytes were found. Analytical column Acquity UPLC BEH C18 (1.7 µm, 2.1 x 50 mm) and gradient elution were used. Optimized composition of mobile phase was acetonitrile : 0.5 mM AmAc pH 4.0 at flow-rate 0.3 ml/min. Electrospray ionization (ESI) in polarity switching mode was employed as an ionization technique. Quantification of all analytes was performed using SRM (selected reaction monitoring). Collision energy and cone voltage were optimized individually for each analyte.

Novel analytical method enabled simultaneous determination of 17 analytes within 10.5 minutes. The validation data indicated good linearity ($r > 0.999$ for allanalytes), sensitivity (LOQ 5 ng/ml for most analytes), repeatability of retention time (< 1.0 % RSD for all analytes) and repeatability of peak area (< 7.0 % for all analytes).

The universal UHPLC-MS/MS method for simultaneous determination of statins, their metabolites and interconversion products was developed and validated. Suitable sample preparation technique was chosen, developed and validated. This method was applied to the real sample of human serum and used for the monitoring of drug levels in human serum of patients treated by statins (especially atorvastatin and rosuvastatin).

The authors gratefully acknowledge financial support of the MSMT of Czech republic - FRVSNo. 894/2012.

PWe-145
11:10 – 12:20

Electrochemical generation of oxidative and reductive metabolites of selected drugs and their UHPLC/MS/MS characterization

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Keywords:
Drug metabolism ; Phase I and II metabolites ; UHPLC/MS ; Electrochemistr ; Anthelmintics.

Novel aspects:
Electrochemical generation of oxidative and reductive metabolites for anthelmintics and other selected drugs, their UHPLC/MS/MS characterization and comparison with in vitro and in vivo experiments.

Abstract:
The study of drug metabolism is a crucial task in the pharmaceutical research. The detection and structure elucidation of Phase I and II metabolites of new drugs is an important step in the drug research and development. In vitro and in vivo experiments are performed for the simulation of oxidative or reductive metabolism using cell cultures and laboratory animals, but the on-line or off-line electrochemical generation of metabolites can be also useful for this purpose. Glassy carbon or precious metal (Pt, Au, Ag) electrodes are used as working electrodes in the electrochemical generation of oxidative products, while the mercury electrode is preferred for the generation of reductive products. The novel laboratory-made design of high-capacity electrochemical cell will be described.

In our experiment, the combination of UHPLC separation with subsequent high mass accuracy measurements in full scan and tandem mass spectra modes using QqTOF analyzer is used for the identification and structural characterization of reductive metabolites of anthelmintic drug flubendazole. The carbonyl reduction leads to the formation of a chiral center, which requires the chiral separation is necessary for the determination of both enantiomers. On the other hand, individual glucuronides of these phase I reductive metabolites are diastereizomers and can be separated using reverse-phase UHPLC.

Concerning the data interpretation, the elemental composition of present metabolites is determined based on the combination of high mass accuracy, specific mass defect, characteristic neutral loss scans, the comparison of fragmentation with the parent drug or relative retention shifts. UHPLC/MS/MS detection of chromophore-containing metabolites in the complex mixture is improved by the parallel UV detection. Advanced software tools are applied for the metabolite identification using the comparison of the blank chromatogram with the electrochemically treated samples. Moreover, the multistage mass spectra measurement using an ion trap based analyzer with the direct infusion introduction of selected metabolites after their isolation provides useful information about their fragmentation patterns. The similar approach is used for the characterization of oxidative products of widespread hypolipidemic drug atorvastatin. The addition of glucuronic or sulphuric acid together with a suitable catalyzer for the generation of corresponding conjugates of phase I metabolites is further studied. Electrochemically generated products are compared with in vivo and in vitro experiments. Finally, the possibility to semi-preparative isolation of drug metabolite standards and their usage for the chromatographic quantitation or their NMR characterization are discussed.

Financial support of this project was provided by the Czech Science Foundation (No. P205/10/0217 and No. P206/12/P065) .

PWe-146
13:30 – 14:40

In-depth pharmaco-phosphoproteomics using meter-long monolithic columns to evaluate molecular-targeting drugs

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Keywords:
Pharmaco-phosphoproteomics, dimethyl labeling, monolithic columns, drug discovery

Novel aspects:
Pharmaco-phosphoproteomic approach with the combination of meter-long monolithic columns and stable isotope dimethyl labeling enabled to discover and validate novel kinase-targeted drugs in a high-throughput manner.

Abstract:
In current cancer treatment, many "molecular-targeted drugs " are clinically utilized and have become major choices of chemotherapy due to the effectiveness instead of traditional anti-cancer agents, and about half of molecular-targeted drugs were designed to target oncogenic kinases which cause aberrant cellular signaling. Protein phosphorylation is well known to regulate diverse cell functions, and therefore, the abnormal profiles are closely related to the onset and development of various diseases including cancers. Thus, the agents which suppress specific protein phosphorylation are thought to be potential candidates for anti-tumor drugs. In order to evaluate the drug candidates efficiently, we should have a grasp of the whole network consisting of kinase-regulated phosphorylation and the alterations in pathological states. In addition, high-throughput method is required for screening candidates. From the above-mentioned points of view, pharmaco-phosphoproteomic approach is currently the most suitable for evaluating kinase-targeted anti-cancer drugs.

Recent advances in phosphopeptide enrichment methods, isotope-labeling strategies for quantitation, MS technologies such as sensitivity and scan speed, and computational algorithms for determination of peptide sequence and phosphorylation sites, are making revolutionary changes in phosphoproteome analysis. Although it is not so difficult to identify/quantify tens of thousands of phosphopeptides by the current approaches, the long analysis time as well as huge amount of samples are inevitably required. In this study, we introduced a stable-isotope dimethyl labeling not only for quantitation but also for enhancing the identification efficiency. In addition, we employed a meter-scale monolithic silica capillary column to provide extremely high identification rate against time.

With dimethyl labeling, 1824 phosphorylated peptides were identified from 600 µg of proteins, whereas 1654 phosphopeptides were identified without dimethyl labeling. In dimethylated samples, we observed more multiply phosphorylated peptides and more phosphopeptides with C-terminal lysine residues, compared with non-labeled samples. These results would be caused by that dimethyl labeling possibly stabilizes positive charges of N-terminal and C-terminal lysyl amino groups and enhances the ionization efficiency, especially for multiply phosphorylated peptides which is difficult to detect in positive ion mode. Furthermore, phosphopeptide enrichment efficiency with titania-based hydroxy acid-modified metal oxide chromatography (Ti-HAMMOC) was improved from 95 to 98%. This small change was quite important because a very small amount of non-phosphorylated peptides dramatically affects the detection limit of phosphopeptides. These results clearly demonstrated that dimethyl labeling is effective not only for quantitation but also for identification efficiency.

In order to perform extremely sensitive and timesaving analysis, we employed a one-dimensional LC-MS system with meter-scale monolithic silica capillary columns and combined two database search algorithms : Mascot™ and ProteinPilot™. As a result, using 125 µg of cell lysates, we have successfully identified about 10,000 phosphopeptides which would be the highest coverage in a single shotgun analysis and, compared to a conventional particle-packed column system, 3-fold increase in MS sensitivity was observed due to the reduced co-elution of digested peptides.

Finally, we combined the above-mentioned techniques to realize high-throughput, comprehensive, and precisely quantitative phosphoproteome analysis, and applied to quantitate the phosphorylation profiles between the cells in basal and drug-stimulated states. A wide range of small molecules, mainly anti-cancer drugs, were administrated to various cancer cell lines, and the phosphorylation profiles were comprehensively quantified and integrated. From these results, we performed cluster analysis to extract the drug-type or cell-type specific alteration, and additionally estimated the unknown signaling networks related to anti-tumor effect. The integrated database is very useful for hunting and validating novel molecular-targeted drugs.

PWe-147
11:10 – 12:20

A Semi-Automated Method for Sequencing Oligonucleotides using ISD and Pseudo-MS3 on a MALDI-Ion Trap-TOF Mass Spectrometer

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Keywords:
MALDI, in-source decay (ISD) , oligonucleotides, ion trap-TOF

Novel aspects:
In-source decay (ISD) and pseudo-MS 3 sequencing of modified oligonucleotides on a MALDI-ion-trap-TOF mass spectrometer

Abstract:
The advantages of MALDI-TOF for the analysis of oligonucleotides include the high sample throughput and the ability to confirm the molecular weight of the intact sample. As a result, MALDI-TOF has been widely accepted for this application. Another advantage of performing oligonucleotide analysis on a MALDI-TOF instrument is the ability to generate sequence information using in-source decay (ISD) , an experiment which can be performed even on the simplest MALDI instrument configuration i.e. linear mode. However, analysis in linear mode, particularly ISD analysis which involves the use of elevated laser powers, typically limits resolution and mass accuracy.

In the case of unmodified RNA oligonucleotides, this may not be a significant problem as the smallest mass difference between unmodified residues is several mass units. However, for oligonucleotides being developed for use as therapeutics, the RNA nucleosides may be modified to increase stability through resistance to degradation. In the case of 2'-O-methyl phosphorothioate-modified RNA oligonucleotides, the mass difference between residues can be as small as 1 Da (modified C = 336 Da, modified U = 335 Da) . In such cases, the lower mass accuracy of linear mode ISD analysis may not be sufficient to unambiguously determine the sequence.

To overcome this, we have performed ISD analysis of the modified oligonucleotides in reflectron mode on a MALDI-ion trap-TOF mass spectrometer. An advantage of the ion trap-TOF instrument is that high resolution and mass accuracy can be achieved across all modes of MS, even when using higher laser power. As a result, monoisotopic resolution was observed for the ISD fragments. One limitation of this approach on this instrument is that fragment ion sensitivity decreases with increasing mass and it was not possible to sequence the entire oligonucleotide in one direction. However, in 2 of the 3 samples studied, sequencing from both ends of the oligonucleotide allowed the complete sequence to be determined.

Sequencing was performed in a semi-automated manner using software originally developed for copolymer analysis. By specifying the formula of the different 'components' of the oligonucleotide (e.g. 4 modified nucleosides, modified phosphate group, end groups, adduct) , the software is used to calculate the masses of various computations within user-defined limits. The high mass accuracy of the MALDI-ion trap-TOF and the observed isotopic pattern can be used by the user to eliminate incorrect compositions. In this proof-of-principle work, sequencing is performed in a sequential manner, increasing the sequence one residue at a time. In future, this process could be fully automated. Using this approach, the *composition* of the 3'- and 5'-ends could be proposed based on the first fragments detected. However, the sequence order of this 3-4 residue tag could not be determined from the ISD spectrum due to the complexity of the spectrum below 1000 m/z. To overcome this, we performed pseudo-MS 3 i.e. MS/MS of an ISD fragment, in an attempt to determine the sequence of the terminal residues.

PWe-148
13:30 – 14:40

Caco-2 PERMEABILITY STUDIES AND DETERMINATION BY LC/MSMS OF MEMANTINE

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Keywords:
memantine, permeability,caco- 2 cell line, LCMSMS

Novel aspects:
BCS Classification of Memantine API's used for development of new generic formulations was identified and Caco- 2 studies of Memantine was used first time to prepare of its biowaiver file.

Abstract:
Memantine is the first in a novel class of Alzheimer's disease medications acting on the glutamatergic system by blocking NMDA glutamate receptors. Despite years of research, there is little evidence of effect in mild to moderate Alzheimer's disease.
We aimed the study to show BCS Class of *Memantine* for its biowaiver file.
Caco- 2 Permeability Studies were performed in the Cell Culture and In Vitro Screening Laboratory of *Abdi Ibrahim Pharmaceuticals R&D Center*.
Caco- 2 cells (ECACC) were seeded at a density of 8.000 cells/well on tissue culture-treated polycarbonate filters (area 0,143 cm 2) in Corning 96 well Transwell plates (Corning Costar) . The culture medium (EMEM) , pH 7.4 containing 10 % fetal bovine serum and 1 % non-essential amino acids (Sigma) , added with penicillin- streptomycin solution (Sigma) was changed every day and cell cultures were incubated at a temperature of 37°C in an atmosphere of 95% relative humidity, 10% CO2. Filters were used for transport studies 21 days after seeding.
Viability and integrity tests were performed by Lucifer yellow test and RTCA (Real Time Cell Analyzer) .
Samples were added into wells at 100 uM concentrations in HBSS (Hank's Balance Salt Solution) containing HEPES buffer at Fassif Mod condition. While incubating cells at 37 °C, aliquots were taken from both chambers at the end of 30- 60- 90 and 120 minutes. Samples diluated in double distillated water and concentrations of compound in the samples were measured by LC-MSMS instrument (Shimadzu LCMS-8030) .
Two different API were used from two different companies and permeability constant of API A was found (P_{aap} 1,283x10^{-5} cm/s) and B was found (P_{aap} 6.63x10^{-6} cm/s) and it is BCS classification was shown as Class I. The difference between two permeability constants of *Memantine* API's could be related to its particule size and polymorphs.

PWe-149

11:10 – 12:20

Development of mass sepctrometric method for pharmacokinetic study of Herceptin

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Keywords:
Herceptin,HPLC-MS/MS,pharmacokinetics

Novel aspects:
We developed a senstive and quantitative mass spectrometric method for Herceptin without using Ab for the potential application of pharmacokinetic studies for protein drugs.

Abstract:
The therapeutic monoclonal antibody drug Trastuzumab (INN ; trade name Herceptin) is widely used for treating metastatic breast cancer patients with overexpression of HER 2 on the tumor. In this study, a sensitive and specific high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was developed and validated for the pharmacokinetic assay system with an enzyme-linked immunosorbent assay (ELISA) system.
Blood sampling was repeated 3 times each for 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 1 day, 2 day, 4 day, 6 day, 9 day, 12 day, 15 day and 30 day after dose. To precisely and quantitatively measure the absolute levels of Herceptin in serum, the proteolytic peptides with Herceptin-specific amino acid sequences were synthesized with incorporated stable isotopes, as ideal internal standards to mimic native peptides formed by proteolysis. The tryptic peptides of serum with standard peptides were subject to analysis with linear trap quadrupole mass spectrometer equipped with a nano-electrospray ion source and quantified by selected reaction monitoring (SRM) mode. The method was sensitive with the lowest limit of quantification (LLOQ) at 0.5ng/ml serum. The dynamic range of the analysis was from 250 fmol/ul to 1 pmol/ul (r^2=0.99) .

PWe-150
13:30 – 14:40

Caco-2 PERMEABILITY STUDIES AND DETERMINATION BY LC/MSMS OF THIOCTIC ACID

Murat Onul[1], Seval Korkmaz[2], Banu Erdem[3]

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Keywords:
thioctic acid, lipoic acid, Caco- 2 cell line, permeability, LCMSMS

Novel aspects:
Usage of LC/MSMS techniques to achieve formulation studies of thioctic acid by improving its permeability results obtained from Caco- 2 studies.

Abstract:
Lipoic acid (LA) also known as *Thioctic acid* and *α-lipoic acid* is an organosulfur compound derived from octanoic acid. LA contains two vicinal sulfur atoms (at C 6 and C 8) attached by a disulfide bond and is thus considered to be oxidized (although either sulfur atom can exist in higher oxidation states) . The carbon atom at C 6 is chiral and the molecule exists as two enantiomers R- (+) -lipoic acid (RLA) and S- (-) -lipoic acid (SLA) and as a racemic mixture R/S-lipoic acid (R/S-LA) . Only the R- (+) -enantiomer exists in nature and is an essential cofactor of four mitochondrial enzyme complexes. Endogenously synthesized RLA is essential for life and aerobic metabolism. Both RLA and R/S-LA are available as over-the-counter nutritional supplements and have been used nutritionally and clinically since the 1950s for various diseases and conditions. LA appears physically as a yellow solid and structurally contains a terminal carboxylic acid and a terminal dithiolane ring.
The aim of current study was to show efficacy of Caco- 2 permeability studies for testing of permeability characteristic of immediate release solid oral dosage formulations of formulations of thioctic acid by comparing with permeability characteristic of its API. Permeability Studies were performed in the Cell Culture and In Vitro Screening Laboratory of *Abdi Ibrahim Pharmaceuticals R&D Center*.
Caco- 2 cells (ECACC) were seeded at a density of 8.000 cells/well on tissue culture-treated polycarbonate filters (area 0,143 cm 2) in Corning 96 well Transwell plates (Corning Costar) . The culture medium (EMEM) , pH 7.4 containing 10 % fetal bovine serum and 1 % non-essential amino acids (Sigma) , added with penicillin- streptomycin solution (Sigma) was changed every day and cell cultures were incubated at a temperature of 37°C in an atmosphere of 95% relative humidity, 10% CO2. Filters were used for transport studies 21 days after seeding.
Viability and integrity tests were performed by Lucifer yellow test and RTCA (Real Time Cell Analyzer) .
Samples were added into wells at 100 uM concentrations in HBSS (Hank's Balance Salt Solution) containing HEPES buffer at Fassif Mod condition. While incubating cells at 37 °C, aliquots were taken from both chambers at the end of 30- 60- 90 and 120 minutes. Samples diluated in double distillated water and concentrations of compound in the samples were measured by LC-MSMS instrument (Shimadzu LCMS-8030) .
Caco- 2 permeability characteristic of thioctic acid and its solid oral dosage form were determined by LC/MSMS analysis techniques.

PWe-151
11:10 – 12:20

Chemical modificomics: Alternative analytical platform for efficient biomarker discovery

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Keywords:
proteomics, chemical modifications, omics

Novel aspects:
Significance of searching the chemical modifications on a specific target protein has been demonstrated as a possible novel omics strategy.

Abstract:
Recent strategies in biomarker discovery have relied on mass spectrometry-based global proteomics. However, biomarker discovery from the entire proteome is analogous to *finding a needle in a haystack*. Accordingly, we have attempted to establish a novel omics strategy to facilitate biomarker discovery. The strategy focuses on only an individual peptide or protein, that is closely related to the targeted disease. Chemical modifications of the selected peptide or protein are exhaustively screened. We call this approach *chemical modificomics*. Proteins and peptides are exposed to higher chemical stress during certain physiological events, i.e. increased oxidative stress in degenerative aging disease and higher glucose stress in diabetes mellitus. Therefore, the resulting chemical modifications can provide meaningful information about biological events. In other words, the modifications serve as dosimeters for stress exposure and as diagnostic and therapeutic markers. However, bioactive peptides or proteins associated with specific diseases have been quantified almost exclusively by immunoassay-based procedures, with no concern for possible minute chemical modifications. Hence, critical information could be overlooked or considerable misunderstandings could occur, if the unidentified modifications alter protein functions. Here, I introduce a novel strategy based on a combination of immunoaffinity clean-up and mass spectrometric identification to screen chemical modifications of a specific target peptide or protein.

Angiotensins: Alternative approach for cardiovascular diseases

Angiotensin II (Ang II), the major bioactive peptide in the renin-angiotensin system, is involved in the regulation of cardiovascular homeostasis and the development of various cardiovascular diseases including hypertension, atherosclerosis, and heart failure. Although the contribution of Ang II in each disease remains unclear, oxidative stress is considered a central mechanism, owing to the involvement of reactive oxygen species in numerous signaling pathways of Ang II. However, there has been little attention given to the potential oxidative modifications of Ang II. We found a novel chemical modification of Ang II generated under oxidative conditions. The structure was unique and similar to Ang A, which exhibits a strong vasoconstrictive effect and elevated concentrations in end-stage renal failure. The structural similarity warrants further investigation of its biological activity.

Keratins: Non-invasive assessment of skin aging

Keratins, the main constituents of human skin, have recently been recognized as major target proteins of chemical modifications, since skin is a main barrier of the body against environmental stresses such as UV and chemical agents. However, because of the difficulties associated with keratins' insolubility and handling, few studies have investigated chemical modification of keratins. We have developed a robust method to screen keratin modifications as a combination of non-invasive sampling (tape stripping), simple clean-up, and tryptic digestion (filter-aided sample preparation), and high-throughput MALDI-TOF/MS analysis. Using the method, we have identified sites modified by oxidation with H_2O_2, and found that $Met^{259, 262, 296, and 469}$ in K 1 were good candidate skin aging markers.

Serum albumin：The most promising global stress marker

Human serum albumin (HSA), the most abundant blood plasma protein, has several important functions including maintaining osmotic pressure and serving as a carrier for hydrophobic compounds. Since HSA is circulated through the entire body, this protein is likely subjected to many chemical stresses. We have optimized a robust method to obtain 100% sequence coverage, thereby enabling exhaustive screening of all the chemical modifications of HSA. Through screening of drug-HSA and chemical-HSA adducts, the strategy is expected to contribute to toxicity and risk assessments involving early-stage drug candidates and environmental chemicals, respectively.

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PWe-152
13:30 – 14:40

Metal Modified Nanoparticle as a Probe for Quantitative Analysis of Clodronate Drug by MALDI-MS in Human Plasma

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Keywords:
clodronate, magnetic nanoparticle, quantification, drug development, sample preparation

Novel aspects:
The metal-chelated nanoparticle assay provided effective and rapid extraction and detection of clodronate from human plasma by MALDI-TOF MS.

Abstract:
Clodronate is a bisphosphonates medicine, which is chosen to alleviate bone resorption and tumor bone diseases. In pharmacokinetic studies, many researchers have been reported for metabolism of clodronate in human biofluid. According to the reports, the pharmacokinetic studies of the clodoenate metabolism pointed that the metabolic concentration was ranged from 0.05 to 3 ug/mL in 48-hour duration after taking the medication. Therefore, it is important to develop an effective and rapid assay to evaluate the pharmacokinetic studies of the clodronate in the bio-fluid. However, due to the matrix complexity in biological matrix, these clinical specimens, such as blood serum, plasma, urine, saliva and etc, generally require a sequence of sample treatment steps prior to analysis. In addition, the bisphosphonates is strong ionic compound leading to hydrophilic characteristic that causes the difficulty of extracting it from bio-fluids.

Thus, several methods for extraction of clodronate have been reported. However, these conventional sample pretreatment methods such as calcium precipitation and solid /liquid phase extraction usually required derivation method that is tedious and time-consuming. With the charge affinity between the bisphophonate group and transition metal ion, we reported a magnetic metal-chelating ligand by covalent conjugation of nitrilotriactic acid (NTA) on the magnetic nanoparticle (MNP) for simultaneous enrichment and detection of clodronate. The several different metalions were studied, and the Zn^{+2}-NTA showed the best efficiency. Without the need of sample derivation and elution, the magnetic NTA nanoparticle demonstrated an efficient metal chelated probe compatible to MALDI MS analysis. Attributing to the high surface-to-volume ratio of Zn^{+2}-NTA@MNP and the adjusting PH value of plasma for modification the charge state of clodronate, the extraction of clodronate can be finished rapidly about 10 min with an excellent efficiency. We also use seed-layer preparation method and spiked internal standard to reduce signal fluctuation in MALDI-TOF analysis for quantitation of clodornate in human plasma. Comparing with the drug metabolism and pharmacokinetic (DMPK) study, the LOQ of nanoprobe-based affinity mass spectrometry (NBAMS) assay is 0.04 ug/mL which is better than it of the traditional GC/MS analysis. Good linear range (R^2=0.9992) was also obtained from 0.04~4 ug/mL in plasma. In addition, the intra-day and the inter-day precisions showed coefficients of variance (CV) from a range of 4.1 to 9.5% and 3.5 to 10.5%, respectively. The accuracy of intra-day and inter-day were found to be from a range of 2.5% to 12.2% and 2.8% to 17.5%, respectively. In conclusion, we demonstrated that a metal modified nanoparticle combined with MALDI-TOF MS is a simple, rapid, reproducible and accurate platform for simultaneous enrichment, detection and quantification of small molecular drugs in pharmaceutical metabolism study.

PWe-153
11:10 – 12:20

Caco-2 PERMEABILITY STUDIES AND DETERMINATION BY LC/MSMS OF TRIMEBUTINE

Murat Yayla[1], Seval Korkmaz[2], Banu Erdem[3], Murat ONUL[4]

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Keywords:
trimebutine, Caco- 2 cell line, permeability, LCMSMS

Novel aspects:
Usage of LC/MSMS techniques to improve formulation studies of trimebutine by improving its permeability results obtained from Caco- 2 studies.

Abstract:
Trimebutine is a drug with antimuscarinic and weak mu opioid agonist effects. The maleic acid salt of trimebutine is marketed under the trademark of Debridat, Recutin, Polybutin, or Modulon for treatment of irritable bowel syndrome and other gastrointestinal disorders. The major product from drug metabolism of trimebutine in human beings is nor-trimebutine, which comes from removal of one of the methyl groups attached to nitrogen. Both Trimebutine and its metabolite are commercially available.
The aim of current study to identify Caco- 2 permeability characteristics of trimebutine immediate release solid oral dosage form and suspension form by comparing with permeability chaecteristics of its API to obtain better generic formulation.
Caco- 2 Permeability Studies were performed in the Cell Culture and In Vitro Screening Laboratory of *Abdi Ibrahim Pharmaceuticals R&D Center*.
Caco- 2 cells (ECACC) were seeded at a density of 8.000 cells/well on tissue culture-treated polycarbonate filters (area 0,143 cm 2) in Corning 96 well Transwell plates (Corning Costar) . The culture medium (EMEM) , pH 7.4 containing 10 % fetal bovine serum and 1 % non-essential amino acids (Sigma) , added with penicillin- streptomycin solution (Sigma) was changed every day and cell cultures were incubated at a temperature of 37°C in an atmosphere of 95% relative humidity, 10% CO2. Filters were used for transport studies 21 days after seeding.
Viability and integrity tests were performed by Lucifer yellow test and RTCA (Real Time Cell Analyzer) .
Samples were added into wells at 100 uM concentrations in HBSS (Hank's Balance Salt Solution) containing HEPES buffer at Fassif Mod condition. While incubating cells at 37 °C, aliquots were taken from both chambers at the end of 30- 60- 90 and 120 minutes. Samples diluated in double distillated water and concentrations of compound in the samples were measured by LC-MSMS instrument (Shimadzu LCMS-8030) .

Caco- 2 permeability characteristic of trimebutine and its oral dosage forms were determined by LC/MSMS analysis techniques.

PWe-154
13:30 – 14:40

Caco-2 PERMEABILITY STUDIES AS A NEW ALTERNATIVE MODEL TO BIOEQUIVALENCE AND BIOWAIVER TESTS

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Keywords:
Caco- 2 cell line, permeability, bioequivalence, biowaiver

Novel aspects:
A novel developed in vitro Caco- 2 permeability studies will be presented as the alternative method to bioequvalence and biowaiver studies..

Abstract:
Frequently, bioequivalence studies of novel developed immediate release oral generic drug formulations can be failed in spite of their successful in vitro results. That dramatic results are related inconcideration of its permeability characteristics according to BCS (Biopharmaceutical Classification System) . The scientific concept of the BCS was incorporated in the regulation of the USA (Guidance for Industry. Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System in the Europena Union regulation (EMEA Committee for medical products for human use CHMP BCS-based biowaiver) and also in the World Health Organization (WHO) . The BCS-based biowaiver approach is meant to reduce *in vivo* bioequivalence studies, i.e., it may represent a surrogate for *in vivo* bioequivalence. Caco- 2 permeability study is already been used to test permeability characteristics of API (Active Pharmaceutical Ingradient) and its solid oral dosage formulations. A new method was developed to show in vitro permeability characteristics of generic solid oral drug formulations by comparing with original/ reference drug under the same condition (FaSSIF) with in vivo bioequivalence tests. Because the results of current developed method were shown similarity with clinical bioequivalence test, it can be proposed as an alternative method for biowaiver files and in vivo bioequivalnece tests (Its patent application has been done) . It has been exerted on usage of Caco- 2 study results to determine of IVIVC (in vitro in vivo correlation) .

PWe-156
13:30 – 14:40

Selectivity test: Potential Interfering substances evaluation during LDTD-MS/MS quantification

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Phytronix Technologies, Quebec, Canada

Keywords:
Small Molecules, Quantitative Analysis, LDTD-MS/MS, Selectivity test

Novel aspects:
Evaluation of potentially matrix interferences substances on drug's quantification using a none chromatographic approach : LDTD-MS/MS.

Abstract:

Introduction

The Laser Diode Thermal Desorption is a rapid analysis approach in which samples are thermally desorbed by a laser diode. Molecules are channeled, using a carrier gas, to a corona discharge region for ionization prior detection via a mass spectrometer. Drugs of interest as well as other potential interfering substances are desorbed at the same time during analysis. To verify the potentially interference effect, the selectivity test is perform using the following molecule (Caffeine, Ibuprofen, Nicotine, Warfarin and Acetaminophen) . They were spiked at the maximum expected concentration in QC sample of a drug of interest (Dextrorphan) . All QC are compared to the reference to evaluate the potential effect.

Method

The following potentially interfering compounds were spiked, at 1 and 10 times the Cmax value reported in literature, in a plasma QC sample containing Dextrorphan : Caffeine at 6 mcg/ml, Ibuprofen at 35 mcg/ml, Nicotine at 30 ng/ml, Warfarin at 340 ng/ml and Acetaminophen at 150 mcg/ml. Those compounds were spiked individually in plasma QC. These potential drug interferences are evaluated on active compound extracts using three different extraction techniques. Protein precipitation : 25 uL of plasma, 50 uL of IS in Acetonitrile and 25 uL of water saturated in NaCl. Liquid-Liquid extraction : 25 uL of plasma, 25 uL NaOH 0.1 N, 10 uL of IS in ACN and 100 uL of EtAc. Solid phase extraction is performed on Oasis HLB (1 cc) cartridge.

Results

Linearity of Dextrorphan expressed by r^2=0.9986 cover a range between 2.5 to 500 ng/ml for the protein precipitation approach. QC samples spiked at 100 ng/ml are compared with potentially interfering substances QC in a protein precipitation extraction. Variations are within 96.5 to 110.4 % range. Liquid-Liquid extraction linearity shows r^2=0.9967 over the same dynamic range. Interfering QC variations are within 94.6 to 108.6%. Solid phase extraction linearity shows r^2=0.9976 over the same dynamic range. Interfering QC variations are within 85.6 to 106.8 % . No analytical difference is observed between the low and high level of interfering substances. Qualification of the method in term of linearity, reproducibility and accuracy meet validation requirements. All measurements of QC fall within the +/- 15% of the expected value which demonstrate the method selectivity on this potential interfering substances test.

PWe-157
11:10 – 12:20

Intact Monoclonal Antibody Characterization Using A Bench-Top Orbitrap Mass Spectrometer

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Keywords:
Orbitrap, Q Exactive, monoclonal antibody, top-down

Novel aspects:
Intact monoclonal antibody characterization using novel scan functions on a bench-top Orbitrap mass spectrometer.

Abstract:

Introduction

Monoclonal antibodies (mAbs) are increasingly developed and utilized for the detection and treatment of diseases including cancer. Due to the heterogeneity of mAb products, thorough characterization is necessary for their reproducible as well as safe production. Among the analytical tools used for the analysis of therapeutic mAb, mass spectrometry (MS) , especially high resolution and accurate mass MS, has become more and more important in providing accurate information on various protein properties, such as intact molecular mass, glycosylation form distribution, amino acid sequence, post-translational modifications, minor impurities due to sample processing and high order structure, etc. In this study, an orbitrap based LC-MS workflow solution was developed for robust, accurate and comprehensive mAb characterization at intact protein level.

Methods

Two different mAbs were analyzed. Intact or reduced mAb samples were desalted, and the light and heavy chains separated using a ProSwift reverse-phase monolithic column. Proteins eluted from the column were analyzed using a bench-top Q Exactive quadrupole Orbitrap or a prototype second generation Exactive Orbitrap mass spectrometer. Top-down MSMS was performed using high energy collision dissociation with a unique spectrum multiplexing feature (msx HCD) in the Q Exctive instrument. Full MS spectra of intact or reduced mAb were analyzed using Protein Deconvolution 1.0 that utilizes the ReSpect algorithm for molecular mass determination. The masses were compared to the expected masses with the various combinations of commonly found glycoforms. The top-down msx HCD spectra were analyzed using ProSight PC 2.0.

Results

The full MS spectra of the mAbs show a complete charge envelope distribution of the mAb, with each charge state revealing five major glycosylation forms that are baseline separated. The measured ppm error on average molecular mass for data generated in 30 experiments using two different instruments is 6.9 ppm with a standard deviation of 6.4 ppm. Using the 140 K resolution setting, the isotopic peaks of the light chain was baseline resolved over the 1 min elution time, which resulted in monoisotopic molecular mass determination with an error of less than 5 ppm. For the top 5 glycoforms, the relative abundance reproducibility was within a few percent. These results present the mass accuracy and reproducibility for mAb intact mass measurement using the bench-top Orbitrap MS instruments in conjunction with the deconvolution software. To obtain amino acid sequence, top-down MS/MS was applied to the reduced mAb samples using the msx HCD approach. In this data acquisition mode, fragment ions produced from several individual HCD events, each on a precursor of a different charge state of the reduced mAb, were detected together in the Orbitrap mass analyzer. Besides the improved throughput from spectrum multiplexing, the advanced signal processing provides improved resolution and higher Orbitrap scan speeds, which is critical for on-line protein top-down sequencing. High resolution, information rich spectra were generated on the one minute LC elution time for reduced mAb samples. For the light chain, over 40% sequence coverage was achieved, including the N-terminal variable region, with a mass error of less than 5 ppm for fragment ions. Results from this study indicate that both precise mass measurement and extensive, high confidence sequence information can be obtained for intact mAb using this workflow solution that combines high resolution MS, fast chromatography, high throughput msx HCD and accurate data analysis.

PWe-158
13:30 – 14:40

Novel approaches in the imitation of oxidative drug metabolism by electrochemistry-mass spectrometry

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Keywords:
electrochemistry, drug metabolism, cytochrome P450, stable isotope labeling

Novel aspects:
Electrochemical synthesis of specific drug metabolites with (on-line) mass spectrometric detection and characterization allows imitation and insight into Cytochrome P450 oxidation reactions

Abstract:
The combination of electrochemistry (EC) with mass spectrometry (MS) forms a powerful analytical tool which finds applications in the modification and characterization drug compounds as well as proteins.
Instrumental methods for the in vitro mimicry of oxidative drug metabolism by Cytochrome P450 enzymes in the body are of great interest for drug development and testing. EC-MS as an analytical technique has unique applications in the field of drug metabolism and may help speed up drug development, first by allowing rapid assessment and prediction of potential metabolites formed in the body, and second by providing a system for the synthesis of sufficient material for subsequent toxicity tests.
In our approach, electrochemical methods are employed to induce either direct oxidation or the formation of intermediate reactive (oxygen) species. The model compound in this study is lidocaine, a local anesthetic, which is metabolized in the liver by CYP450 isoenzymes to its N-oxide and to several hydroxylated and N-dealkylated forms. In an electrochemical cell, direct oxidation at glassy carbon electrodes is very efficient and selective for the N-dealkylation reaction, but is not capable of the other important oxidation reactions such as aromatic hydroxylation. Alternatively, reactive oxygen species can be electrochemically produced in a controlled way by oxygen reduction or by the electrochemically induced Fenton reaction. Strict control of instrumental parameters, such as potential, square-wave pulsing with variable cycle times, electrode material and solvent conditions, are used to tune the type and distribution of oxidation products, and we have developed a set of conditions which will allow reliable and controlled oxidative modification of model drug compounds, based on the available functional groups and desired oxidation products.
The oxidation products of drug compounds are often isobaric, due to introduction of an oxygen atom in different places in the molecule. The use of stable isotope-labeled reagents (^{18}O-labeled water, or hydrogen peroxide) and specific fragmentation methods (thermal decomposition of N-oxides during APCI) enabled the correct assignment of isobaric oxidation products. In addition, the stable isotope method allowed the identification of the source of the oxygen atom in each product (molecular oxygen, water or hydrogen peroxide) , which provides valuable insight in the electrochemical and chemical reaction mechanisms. The mechanisms proposed for the EC-induced modifications are compared with the known CYP450 catalyzed reactions.

PWe-159

11:10 – 12:20

Analysis of lipid composition in stimulated human cancer cells by LC-MS/MS following treatment with protolichesterinic acid isolated from Cetraria islandica

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Keywords:
LC-MS/MS, Eicosanoids, Lipoxygenase, Lichen, Chemometry.

Novel aspects:
Analysis of the effects of protolichesterinic acid isolated from Cetraria islandica by targeted lipidomics in stimulated human cancer cells.

Abstract:
Cancer cells show differences from healthy normal cells in their metabolism, which contribute to their survival and growth. Lipids have numerous functions in biological processes, structural as well as regulatory. Fatty acid synthase (FAS) is an essential enzyme for cancer cells and a high level of FAS is a characteristic of many human malignancies. Hydroxyeicosatetraenoic acids (HETEs) the products of lipoxygenase (LOX) pathways have been implicated in cancer development. Protolichesterinic acid (PA) , a secondary metabolite of lichens, is a potent inhibitor of 5- and 12-LOX and may also have FAS inhibitory activity. The aim of this work was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for evaluation of lipid composition in stimulated human cancer cell lines, before and after treatment with PA. PA was purified from a petroleum ether extract of *Cetraria islandica* with preparative high performance liquid chromatography (HPLC) . A LC-MS/MS method was developed and optimized for quantification of palmitic acid, HETEs and LTB_4, utilizing a chemometric approach. Seven variables ; type of column, flow rate, initial amount of organic solvent, type of organic solvent, gradient slope, collision energy and capillary voltage were optimized by D-optimal design and related to sensitivity, retention time and resolution utilizing PLS-regression. Different sample preparation methods ; solid phase extraction, protein precipitation and liquid-liquid extraction were tested and parameters optimized. The amount of HETEs and the derivative, LTB_4 in both cultured cancer cells and cultured medium were analyzed with the optimized LC-MS/MS method after stimulation with calcium ionophore (A23187) and treatment with PA. Preparative HPLC was successfully used for crude-purification to obtain one of the major secondary metabolites in the lichen *Cetraria islandica* (L.) Ach., PA and its tautomer lichesterinic acid. NMR and analytical HPLC were used to verify the purity and identification of PA. The identification was confirmed and the purity was evaluated as 99.60%. Palmitic acid could not be successfully analyzed because of contamination problems and extraordinary precautions need be taken to achieve accurate analysis of palmitic acid and to avoid fatty acid contamination. Identification and optimization of significant experimental factors influencing the response for the LC-MS/MS method were done by design of experiments (DoE) . The sensitivity was significantly influenced by flow rate, collision energy, gradient slope, organic phase and type of column. Interaction between gradient and organic phase was significant which indicated that these variables could not be independently controlled to obtain optimum conditions. A LC-MS/MS method was developed and validated for quantification of the LOX pathway products ; LTB_4, 5- and 12-HETE. An intraday validation assessment showed that the quantitative determination was linear for LTB_4, 5- and 12-HETE in the range tested (0.120-30 ng/mL) , and accuracy and precision met the acceptance criteria with a coefficient of variations lower than 15%. Degree of interference was assessed and the conclusion was that no significant interfering peaks were detected. Stability of the analytes was established for 12 hours and storage at 4 °C was found to be appropriate. Quantification of LTB_4 and HETEs in the pancreatic cancer cell line Capan-2, before stimulation with A23187 showed a weak signal (approximately 10-300 pg) for the analytes, suggesting the presence of these analytes in the cell samples. To confirm the presence of these lipids an optimization of the extraction method for lipids from the cells is needed and is in progress. This will include development of methods, ranging from the initial step of the cell membrane rupture to the final sample preparation method prior to LC-MS/MS quantification of lipids in cultured tumor cells. The tumor cells lines have been stimulated with A23187 and the amount of LTB_4, 5- and 12-HETE will be analyzed with the optimized LC-MS/MS.

PWe-160

13:30 – 14:40

UHPLC-MS-MS with Precursor Ion Scanning, Neutral Loss Scanning and Polarity Switching for the Detection of Glutathione Conjugates of Reactive Metabolites

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Keywords:

drug metabolism, UHPLC-MS-MS, glutathione, fast triple quadrupole, stable isotope labeling

Novel aspects:

Unique combination of polarity switching, UHPLC, isotope labeling, precursor ion scanning, and constant neutral loss scanning with an ultrafast triple quadrupole for the screening of complex mixtures for glutathione conjugates.

Abstract:

Drugs, compounds undergoing drug development, and natural products contained in botanical dietary supplements are sometimes metabolized to electrophilic metabolites. These reactive metabolites can produce cytoxic or even genotoxic effects such as oxidative stress, covalent modification of proteins and DNA. In vivo, reactive metabolites can be deactivated through reaction with the endogenous nucleophile gluathione, and glutathione conjugates can be detected and characterized using positive ion tandem mass spectrometry or negative ion tandem mass spectrometry. Drawing on this information, our laboratory (van Breemen et al., Comb Chem High Throughput Screen, 1999) reported a positive ion electrospray mass spectrometric screening method for the rapid detection of reactive metabolites formed during in vitro incubations of drugs with human drug metabolizing enzymes, enzymatic cofactors, and glutathione. Subsequently, Dieckhaus et al. (Chem Res Toxicol, 2005) reported the use of negative ion electrospray tandem mass spectrometry for the detection of glutathione conjugates that did not form abundant positive ions, and Yan and Caldwell (Anal Chem 2004) reported the use of dual intrinsic labeling of glutathione to eliminate false positive results during tandem mass spectrometric screening of complex matrixes for glutathione conjugates. Using a new generation fast triple quadrupole (Shimadzu LCMS-8040) , we have succeeded in combining our original positive ion screening approach with the negative ion method and the stable isotope labeling method into a fast and efficient UHPLC-MS-MS assay. During a single UHPLC separation, positive ion electrospray constant neutral loss scanning for the elimination of a characteristic gamma glutamyl group from an unlabeled region of glutathione is measured alternately with negative ion electrospray precursor scanning for the characteristic glutathione anions of *m/z* 272 and its isotope labeled form of *m/z* 275. This new assay detected more glutathione conjugates than was possible using either the positive ion method or the negative ion method alone while eliminating false positive results. Implementation of UHPLC facilitated faster separation and detection of glutathione conjugates than previous methods. The use of an ultrafast scanning triple quadrupole capable of rapid polarity switching was essential not only because of the multiple types of MS/MS scans but also because of the UHPLC time scale which was 10-fold faster than previous HPLC studies. This new glutathione conjugate detection method was validated using drugs such as acetominophen that are known to form electrophilic quinone imine metabolites. Then, the method was used to screen popular botanical dietary supplements that are used by menopausal women as alternatives to hormone replacement therapy. For example, following incubation of a botanical extract prepared from *Glycyrrhiza glabra* (licorice) with human liver microsomes, NADPH and glutathione, the natural product glabridin was identified as a constituent of licorice that can be metabolized to reactive intermediates that form glutathione conjugates. This new UHPLC-MS-MS approach to screen complex mixtures of drug metabolites and botanical extracts for the potential to form electrophilic metabolites may be used to complement safety studies of dietary supplements and drugs under development.

PWe-161 Comparison of titanate nanosheets by G-SIMS and g-ogram

11:10 – 12:20

Ichiro Mihara[1], Satoka Aoyagi[2], Keizo Nakagawa[3]

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Keywords:
G-SIMS, g-ogram, TOF-SIMS, Titanate nanosheet

Novel aspects:
Fragment ions from organic-inorganic complex samples were characterized by G-SIMS and g-ogram to evaluate their chemical structures in nanolayers.

Abstract:
Nanostructure evaluation is important for developing highly controlled nanomaterials. Layerd titanate nanosheets having a lamellar mesostructure were analyzed using time-of-flight secondary ion mass spectroscopy (ToF-SIMS) in order to evaluate detailed chemical structures. Although ToF-SIMS is a useful method for evaluating complex organic and inorganic materials, it is often difficult to interpret ToF-SIMS spectra of complex samples due to intricate fragment ions mainly from organic materials. Recently Gentle-SIMS (G-SIMS) and g-ogram have been developed in order to interpret complicated secondary ions in static-SIMS spectra. Secondary ions are classified based on ratio of static-SIMS spectra obtained with two primary ion conditions causing different degrees of fragmentation. The g-ogram is a derivative technique from G-SIMS and provides useful information on origins of secondary ions.

In this study, G-SIMS and g-ogram were applied to organic-inorganic complex to characterize secondary ions related to organic materials and to distinct molecular ions and more intact fragment ions from fragment ions of larger molecules. Several layered titanate nanosheets samples produced were prepared using different surfactants, dodecanediamine (DDA) , laurylamine (LA) and hexylamine (HA) , as templates to produce lamellar structures. TIPT + DDA + TEOA, TIPT + LA and TIPT + HA samples were prepared using tetraisopropyl orthotitanate (TIPT) , triethanolamine (TEOA) and DDA, and were prepared using TIPT and surfactants, laurylamine (LA) and hexylamine (HA) , respectively. As a result, secondary ions related to titanate compounds were detected in the titanate nanosheet samples having lamellar mesostructures though they were not detected in TIPT samples without surfactants. Especially in TIPT + DDA + TEOA sample the surfactant molecular ion was also observed, and therefore it is indicated that robust lamellar mesostructure of titanate compounds and the surfactant is produced by reactions related to TIPT, DDA and TEOA. It is also suggested that organic-inorganic materials which have large hydrocarbon groups can be analyzed using G-SIMS.

PWe-162
13:30 – 14:40

Adaptive Thresholding for Three-Dimensional Wavelet Denoising of TOF-SIMS Images : Toward Digital Staining of Pathological Specimens

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Keywords:
three-dimensional wavelet analysis；adaptive thresholding

Novel aspects:
We propose an adaptive three-dimensional wavelet algorithm for rapidly denoising data, and it determines the wavelet threshold based on an optimum thresholding by gradually changing a temporarily set threshold.

Abstract:
This report presents a novel noise reduction algorithm for spatially distributed mass spectrometric data. In recent years, mass spectrometry imaging has received considerable attention, owing to its capability to monitor many molecules at the same time. Mass spectral peaks corresponding to particular molecules can be used as biomarkers in disease diagnosis. We have recently focused on time-of-flight secondary ion mass spectrometry (TOF-SIMS) , which enables two-dimensional mass spectrometry imaging of components with sub-micrometer resolution. In mass spectrometry, noise reduction is crucial for identifying weak peaks. Up to now, various methods for denoising mass spectra have been proposed. Because of the high spatial resolution of TOF-SIMS, the two-dimensional distribution of components, which is reconstructed by using specific *m/z* peaks, can be treated as continuous. Therefore, TOF-SIMS data have, in essence, three-dimensions. A difficulty in TOF-SIMS, however, is that extremely large amounts of data are generated, especially in measurements on pathological tissue samples. Therefore, a fast algorithm is necessary for the practical application of noise reduction to TOF-SIMS data.

We have recently reported an adaptive three-dimensional wavelet algorithm for rapidly denoising data acquired by TOF-SIMS as follows. In the computation, the TOF-SIMS data are stored in three-dimensional space, where the *xy*-plane corresponds to the measured area and the *z*-axis corresponds to the *m/z* mass spectrum. Reconstructed images for specific *m/z* peaks along the *z*-axis indicate the spatial distributions of components such as protein and lipid. In this algorithm, two different basis functions are applied to the *xy*-plane and the *z*-axis, and three-dimensional wavelet shrinkage and reconstruction is performed. One basis function is suitable for continuous data such as the distribution of components, and the other is suitable for discrete data such as a mass spectra. This method has resulted in marked noise reduction of TOF-SIMS data, while it still had arbitrariness in determining the threshold of wavelet analysis.

In this study, we propose an adaptive thresholding algorithm for wavelet denoising. In addition to the different basis functions, we determine the wavelet threshold based on an optimum thresholding by gradually changing a temporarily set threshold to evaluate the effect of the threshold on the noise reduction. To evaluate the effect on the noise reduction, the amount of change in signal before and after the noise reduction is estimated from the amount of change in the standard deviation of the signal. The results show that the noise is reduced without loss of the original signals and that the signal-to-noise ratio is substantially improved by the noise reduction algorithm.

PWe-163
11:10 – 12:20

Elucidating the aberrant PK Profiles of Monoclonal Antibody by Proteomics Approaches

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Keywords:
Monoclonal antibody, pharmacokinetic, tissue analysis,

Novel aspects:
Immunocapture technique based on anti-human antibody to pull down both the drug and target to perform a multiplxed PK/PD analysis.

Abstract:
Monoclonal antibodies (mAbs) have emerged as one of the most important drug classes in the biopharmaceutical industry. An understanding of the factors which can affect the pharmacokinetic (PK) behavior, particularly the elimination mechanisms is pivotal during their pre-clinical and clinical evaluation. Drug ABC exhibits a rapid clearance in Cynomolgus monkey. Deposition of this mAb in lymph node and spleen is observed by recent in-house immunohistochemistry study. However, there is little information available on the extent of ABC accumulation in tissue, the stoichiometry of target and drug ABC deposition and the impact of such accumulation on its PK profile. The purpose of this project is to establish liquid chromatography coupled with mass spectrometry (LC-MS) and the-state-of-art proteomics approaches to quantify drug ABC in serum and tissue samples. In order to increase further the selectivity and sensitivity of LC-MS/MS, an immunocapture technique based on anti-human antibody that allows efficient extraction of human mAb from Cynomolgus monkey serum and tissue is developed. Combined with LC-MS/MS, a multiplexed PK/PD assay within a single analysis is generated

PWe-165
11:10 – 12:20

Mass spectrometry-based non-invasive proteomic analysis of human skin keratins for discovery of biomarkers of oxidative skin damage

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Keywords:
keratins, oxidative skin damage

Novel aspects:
We have developed the first MS-based approach to detecting oxidative modifications in keratins obtained directly from human skin and found the potential biomarkers of oxidative skin damage.

Abstract:
Human skin is one of the major barriers of our body against environmental stimuli such as UV and industrial chemicals. Therefore, as the main constituent of the skin, keratins have recently been recognized as the major oxidative target proteins. However, because of the difficulties associated with their insolubility and handling, there have been lack of studies to identify the sites of oxidation in keratins. Here, we introduce a combination of non-invasive sampling, simple clean-up, efficient digestion method, and mass spectrometric (MS) analysis to screen keratin modifications. Human skin proteins were obtained from the upper arm with adhesive tapes. After the tape stripping, one-fourth of the tape was placed into a tube containing sodium dodecyl sulfate (SDS) buffer and butylated hydroxytoluene (BHT). Proteins on the gluey surface were then solubilized by scraping using a grinding plastic pestle. After the centrifugation, the supernatant was used for the following proteomic analysis. SDS, DTT and IAA were removed at every step by centrifugation with a filter unit (>30 K). The resulted protein mixture was digested by trypsin on the filter unit and the tryptic peptides as filtrate were then analyzed by MALDI-TOF/MS, LC-ESI/MS, and MS/MS. From the human skin proteins obtained by tape stripping, keratines have been identified as major proteins (82 out of 101 peptide peaks). Especially, K1 and K10 have been found as the predominant keratins with sequence coverages of approximately 45%. Most of the keratin methionine sites detected in our experiment were found to be partially oxidized in intact human skin samples. Among them, specific sites, such as Met^{259}, Met^{262}, Met^{296}, and Met^{469} located in the α-helical rod domain of K1, were shown to be the most susceptible to oxidation with hydrogen peroxide. Thus, their relative oxidation levels were increased in a dose- or time-dependant manner upon treatment with hydrogen peroxide in vitro or in vivo, respectively. Therefore, we believe that this approach would provide significant information of the oxidative modifications on keratins that could be useful as biomarkers of the skin damage.

Supported by KOSÉ Cosmetology Grant from The Cosmetology Research Foundation, Japan (to T.O.).
Reference : Lee SH, Miyamoto K, Goto T, Oe T : J. Proteomics, 75, 435-49 (2011).

PWe-166
13:30 – 14:40

RELATIVE QUANTITATION OF TMT-LABELED PROTEOMES FOCUS ON QUANTITATIVE PRECISION AND ACCURACY

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Keywords:
MS3, TMT Quantification, Orbitrap Elite

Novel aspects:
Improvements in TMT based quantification using MS 3 HCD and Orbitrap Elite

Abstract:
Introduction: Isobaric tagging methods involving 'differential' isotope labeling using chemical tags are universally applicable approaches measuring relative amounts of proteins across two or more different samples. The main focus of the presented work was on assessing the quantitative precision and accuracy for a TMT sixplex-labeled complex proteome sample using latest ion trap-Orbitrap hybrid instrumentation.

Methods: Six aliquots of a digest of 9 proteins labeled each with one of the TMT sixplex reagents and mixed to obtain the final ratio 10 : 1 : 10 : 2 : 10 : 1.5. Such sample was added to the background of a TMT sixplex-labeled E. coli lysate digest. Peptides were analyzed using nano-LC coupled to the Orbitrap Elite. A data dependent method (Top10 method) , as well as an MS 3 -based method where MS 3 multipole collision cell fragmentation was triggered on a strong fragment ion from a particular m/z range of an ion trap fragmentation spectrum (MS 3 method) were evaluated. Quantitative precision was expressed as protein ratio variability. Quantitative accuracy was presented as a deviation of a measured
ratio value from the expected value.

Results: The percentage of peptides whose fragmentation spectra contained all six reporter ions (quantifiable peptides) exceeded 90% for any sample load tested. The variability was lower than 10% for approximately 90% of the quantified proteins at a 500 ng sample load, and for about 70 % at a 20 ng sample load. The Orbitrap detection at resolution settings 15,000 corresponds to effective resolution >27,000 for m/z 126-130.Consequently, a very tight mass tolerance (+/- 10 ppm) could be used effectively filtering most reporter ion interferences. For higher ratios (theoretical ratio 10 : 1) the median peptide ratio increased from 5.64 (Top10 method) to 7.97 (MS 3 method) .
Conclusion: An improved mass spectrometer design resulted in a high percentage of quantifiable peptides. The quantitative precision is signal intensity-dependent. Very high resolution effectively eliminated isobaric interferences in the reporter ion region. The impact of peptide precursor co-isolation issue on quantitative accuracy was significantly reduced with MS 3 method.

PWe-167
11:10 – 12:20

Mobility Assisted Data Independent Label-Free LC-MS Analysis of Citrobacter rodentium Infected Mouse Colon

Maki Terasaki[1], Chris Hughes[2], Johannes P Vissers[2], James Collins[3], Gad Frankel[3]

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Keywords:
Label-Free, Quantitation, Ion-Mobility, Data-Independent Analysis

Novel aspects:
Comparative analysis and classification of Citrobacter rodentium infected mice by Mobility Assisted Data Independent Label-Free LC-MS

Abstract:

Introduction

Citrobacter rodentium is a murine specific pathogen used to study human infection with enteropathogenic and enterohaemhorragic *E. coli*. *C. rodentium* colonises the gut via formation of attaching and effacing lesions, causing transmissible colonic hyperplasia and a self-limiting disease in C57Bl/6 mice. In this presentation, we describe the use of quantitative proteomics experiments to determine changes to the murine colonic proteome at two key time points of infection, day 8 (bacterial colonisation peak) and day 14 (colonic hyperplasia peak) . We used liquid chromatography coupled with ion mobility and mass spectrometry to determine relative abundance changes to the colonic proteome. Subsequent experiments focused on the effects of bacterial mutants in key virulence determinants on protein expression in the mouse colon.

Methods

Six week old mice were infected with C. rodentium or administered PBS as a control. At day 8, or day 14 post infection, mice (4 infected, 4 control) were euthanized, 6 cm of colon removed and pooled (2 colons per sample) for protein extraction and trypsin digestion. Peptides were reversed phase separated, using a nanoscale LC system, configured with a trap column and a 75 µm analytical column. A 90 min, 300 nL/min gradient was performed with each sample analysed in triplicate. Data were acquired in HDMSE mode where raising the collision energy following ion mobility separation produced fragments that exhibit the same drift time as their precursor. Precursors and fragments were correlated and searched using post processing software.

Abstract

Initial experiments were conducted to determine the optimum extraction procedure of proteins from mouse colon. Proteins were extracted either using 5 % Rapigest, Molloy-like lysis buffer or Molloy-like lysis buffer in the absence of CHAPS. Samples were digested with trypsin and it was determined, from the number of proteins identified, approximately 850 from 1 μg of starting material, that the extraction method based on Molloy-like lysis buffer was the most optimal. For the comparative study, eight mouse colon tryptic digest samples (four infected, four controls) were each analysed in triplicate and random order, with the amount of material loaded for these experiments reduced to around 100 ng. The resulting data were searched with the UniProt mouse database using a dedicated data independent search algorithm and a total of 2852 proteins comprising at least 1 proteotypic peptide were identified from the entire sample set, with approximately 1000 proteins from 10,000 non-redundant peptides being identified in each injection and over 1250 proteins in two out of the three injections for each sample. Injections of 50 ng *E. Coli* tryptic digest were made at various points to ensure that performance of the LCMS system was maintained throughout the entire experiment. Reproducibility was demonstrated with over 800 proteins consistently identified in each of the QC injections. Further interrogation of the dataset allowed for the calculation of the relative in-sample abundance for each identified protein. Thereafter, unsupervised hierarchical clustering of the relative abundance calculated values demonstrated a clear classification of *C. rodentium* infected and uninfected mice and afforded identification of both host and pathogenic infection related proteins.

PWe-168
13:30 – 14:40

A method for isolation of endogenous disulfide-containing peptides from a biological sample.

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Keywords:
Disulfide bond, Pituitary, 18O-labeling

Novel aspects:
A method for isolation of endogenous disulfide-containing peptides

Abstract:
A disulfide bond is formed between two cysteine residues and deeply involved in protein folding and expression of biological activity. In human and *E. coli* sequence databases, at least 80% of proteins and peptides contain multiple cysteine residues. The fact that many of bio-active peptides have disulfide bonds prompted us to develop a method for selectively isolating these peptides from a complex mixture, which may lead to discovery of a novel bio-active peptide in a biological sample.

Pituitary produces many biologically active compounds such as various peptide hormones, which are produced by several enzymatic cleavages from large precursor proteins, despite that the modes of processing have not been fully understood.

In order to isolate only disulfide-containing peptides, a method using a hetero-bifunctional cross-linker (SPDP : *N*-succinimidyl 3-(2-pyridyldithio) propionate) linked to aminopropyl resin has been developed. The conditions and efficiency of the isolation were examined using synthetic peptides and a tryptic digest of bovine serum albumin, a 69-kDa protein containing 17 disulfide bonds. The protein was reduced under denaturating conditions, cleaved with trypsin, and directly applied to the above resin. The eluate from the resin with a reducing agent was found to predominantly contain peptides with Cys residue (s) , while peptides without Cys were observed in the flow-through.

Peptides in a biological sample are always susceptible to degradation during isolation, which mainly owes to the action of various enzymes present in the sample. We here successfully applied the ^{18}O-labeling method which allows for discrimination of endogenous peptides from the degradation products that are produced during isolation and purification, i.e. the decomposed peptides are distinctively labeled with ^{18}O as the result of hydrolysis in ^{18}O water. Pituitary tissue from *Rattus norvegicus* was put into ^{18}O water and boiled for 10 min to deactivate enzymes present in the sample. Peptides were extracted with 0.25% aqueous acetic acid. The preliminary analysis of a crude extract with MALDI-MS/MS gave non-labeled and ^{18}O-labeled peptide signals, which could be differentiated by the shift in mass of 2 or 4 Da.

A combination of the above two methods should be useful for discovering endogenous disulfide-containing peptides in pituitary tissue, which might be candidates of bioactive peptides with unique structures formed by disulfide bond (s) .

PWe-169
11:10 – 12:20

A Targeted Quantitative Phosphoproteomic Approach using iTRAQ-Labelled Synthetic Peptides as Internal Standards

Andre C Mueller, Leonhard Heinz, Kumaran Kandasamy, Manuela Bruckner, Christoph L Baumann, Giulio Superti-Furga, Keiryn L Bennett

CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences/Vienna/Austria

Keywords:
Quantitative phosphoproteomics, iTRAQ, targeted analysis, synthetic phosphopeptide library

Novel aspects:
The use of iTRAQ-labelled synthetic phosphopeptides in a pseudo-selected reaction monitoring 'targeted approach' as a robust validation method to study signalling networks.

Abstract:
Mass spectrometry (MS) is widely-used to study posttranslational modifications of proteins. Phosphorylation of serine, threonine and tyrosine residues are amongst the most-studied and best-understood of all protein modifications. Continuous improvements in phosphopeptide enrichment strategies and advances in sensitivity, accuracy and speed of MS instrumentation allow the identification of thousands of phosphorylation sites in a high-throughput manner. Phosphoproteomic approaches are used to study phosphorylation events within cellular signalling pathways in the context of innate or adaptive immunity, cancerous cells or tissues. To infer a biological significance for a given scenario, comparative quantitative proteomic approaches are necessary to enable the detection of regulated sites and determine the fold-change in phosphorylation. The use of the iTRAQ 4-plex technology allows the multiplexing of 4 samples in a single mass spectrometric analysis. Thus, the comparison of the relative abundance of a peptide/protein or modified peptide/protein across states is facilitated.

A global phosphoproteomic analysis was conducted to determine the regulatory effects of a newly-identified negative regulator (referred to as 'LiMIT') of pro-inflammatory toll-like receptor (TLR) signalling in murine macrophages. Stable RAW264.7 cell lines with lentivirally-transduced shRNAs either targeting 'LiMIT' expression or non-targeting negative control RAW264.7 cell lines expressing endogenous levels of 'LiMIT' were used for two independent experiments. Lipopolysaccharide (LPS) activated the TLR/MAPK pathway, and a relative comparison of the unstimulated cells with 10 and 20 minute post LPS-stimulation was achieved with the iTRAQ 4-plex labelling strategy. The fourth iTRAQ channel was used for a reciprocal cross comparison of non-stimulated LiMIT knockdown versus LiMIT wild-type and vice versa. One hundred micrograms of total protein per iTRAQ channel was prepared, and the tryptic peptides labelled with the 4-plex reagent. A modified IMAC strategy based on Ficarro *et al.*[1] was utilised to enrich iTRAQ-labelled phosphopeptides. The resultant samples were fractionated with reversed-phase chromatography at pH 10. Ten and fifty fractions were collected for the phosphoproteome and the phospho-depleted proteome, respectively. Samples were analysed in duplicate on an LTQ Orbitrap Velos mass spectrometer using a hybrid multi-stage activation-collision-induced dissociation/higher-energy collision-induced dissociation (MSA-CID/HCD) method for phosphopeptide analysis. The phospho-depleted proteome was analysed by HCD alone.

The data was searched against the murine SwissProt database with MASCOT and evaluated with Proteome Discoverer. Over 3,400 annotated phosphopeptides were grouped into more than 1,900 protein groups. Manual validation is still ongoing ; however, preliminary results have identified at least 91 proteins showing the effect of LiMIT knockdown on the regulation of phosphorylation ; with 26 proteins belonging to the MAPK/TLR network, *e.g.*, ERK or Raf1.

The reproducibility of MS identification and iTRAQ quantitation can vary strongly in data-dependent acquisition mode and therefore a pseudo-selected reaction monitoring (SRM) 'targeted approach' has the potential to provide improved peptide identification and strengthen statistics. From a selected list of differentially-regulated tryptic phosphopeptides, synthetic versions will be produced. These phosphopeptides will be used as universal internal standards for inter-experimental comparison and concurrent peptide validation. The envisaged workflow will consist of : (i) purification of crude synthetic phosphopeptides ; (ii) validation of the peptides by MS ; and (iii) refinement of the MS method for optimisation of peptide fragment ion sequences and concomitant iTRAQ quantitation (dynamic range, ion statistics) . Following proof-of-concept, the 'phosphoproteomic analysis will be repeated with an extended library of iTRAQ-labelled, standard peptides multiplexed with the three cellular states (LPS stimulation at t = 0, 10 and 20 minutes) for the LiMIT knockdown and the control experiment. The ultimate objective of the targeted approach is to implement a generic, technical platform that enables a fast, robust and normalised quantitative proteomic analysis across different experiments.

[1] Ficarro, S. B., *et al.* (2009) *Anal. Chem.,* 81, 4566-4575

PWe-170
13:30 – 14:40

The study of aging processes using in vitro glycation experiments applied to low density lipoproteins using MALDI Mass Spectrometry

Omar Belgacem[1], Matthew Openshaw[1], Grazyna Sobald[2], Katharina Pock[3], Gerald Stuebiger[4]

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Keywords:
MALDI, Glycation, Aging, Lipoproteins, Bioinformatic

Novel aspects:
The detection of glycation sites for Low Density Liporproteins that are located near binding receptors. The detection of unusual amino acid modifications that could be an indication of aging.

Abstract:

Introduction
The aging and oxidative processes are widely known to induce all sorts of health problems that are now causing a huge stress on the healthcare system of any modern country. The glycation process, also known as Maillard adductions, is involved in not only human disease and pathology but also in food processing and pharmaceutical formulations. We have developed over the years a methodology to specifically detect glycation sites on various standard proteins in order to be able to look at real samples such as human serum albumin and Low density lipoproteins (LDL) in vivo. We have also tried to link our findings with LDL lipids profiles.

Methods
Standard proteins and peptides (BSA, Substance P) were used for the method development and the generation of MSMS signatures at the modified peptide level. Glycated (g) , glycoxidated (gox) lipoproteins samples were generated in vitro using a very soft glycation process mimicking physiological conditions. The lipoproteins were obtained by ultracentrifugation of normal human plasma. Proteins were then digested with trypsin. Peptides were separated by HPLC and sequence mapping was performed by MALDI-MS/MS. Mass spectra were recorded using MALDI-QIT-TOF-MS/MS platforms in order to generate the MSMS signatures of the modified peptides. MALDI-TOF-RTOF was used for the LC-MALDI mapping. Data were processed using a software equipped with a database of user-defined post-translational modifications (PTM-Finder™) . The Phospholipids (PLs) were isolated by directly extracting the lipids from the LDL particles using a solvent extraction method. PLs profiles were then directly analyzed by MALDI-MS.

Results
We have designed a method allowing us to specifically detect glycated peptides out of a very complex mixture. This was done by first generating MSMS signature specific to peptides that are glycated with glucose, lactose and maltose. These signatures, which consisted in characteristic neutral losses (-162, -120 and-36 Da) , were independent of the peptide chain but directly linked to the sugar moiety attached to the amino acid backbone. We incorporated these losses into a bioinformatic platform in order to specifically detect glycated peptides. This was done by using Mascot searches in combination with an MSMS screening module (PTM Finder software) . The g- and gox-LDL samples were digested with trypsin and separated through a micro-column in order to avoid any ion suppression effect. Following digestion and LC- separation, it was interesting to note that out of 357 lysine residues ; only two of them were glycated. We were able to cover 33 % of the whole amino acid sequence which account for approximately 120 potential lysine glycation sites. The 2 detected glycations sites are located near binding receptors which may explain some biological dysfunction in diabetic patients. Another interesting aspect is the large number of peptides (MSMS spectra) that did not match any known amino acid sequence of the Apo-B-100. We suspect that these peptides were modified but not glycated and therefore could not be detected using standard bioinformatics approach (Mascot, PTM search etc) . These peptides are now in the heart of a new investigation where the aim is to detect peptides that reacted with highly reactive lipids species within the LDL particles during the oxidation/glycation process. Ultimately the quantifications of such peptides could be a strong indicators of the general health of the patient.

PWe-171
11:10 – 12:20

Phosphoprotein analysis using a combination of an online monolithic trypsin-immobilized enzyme reactor and TiO2 enrichment column with tandem mass spectrometry

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Keywords:
IMER, TiO2, Phosphoprotein, Tandem mass spectrometry

Novel aspects:
We demonstrated the online based combined applications for analysis of phosphoproteins.

Abstract:
Post-translational modifications (PTMs) of proteins entail a change of proteins in chemical compositions by proteolytic cleavage or by addition of functional groups at certain amino acid residues. Many researchers have identified the involvement of the phosphorylation PTMs in many biological pathways or processes. However, the analysis of phosphorylated PTMs is still a formidable task in the biological and technical aspects due to several reasons such as structural homogeneities and its low relative abundances. To overcome the drawbacks, we demonstrated the online based combined applications for analysis of phosphoproteins. The system is composed of three of main part, such as online phosphoprotein digestion using trypsin immobilized enzyme reactor (IMER) , online phosphoprotein enrichment using TiO_2 column, and dual tandem mass spectrometry with collisionally activated dissociation (CAD) and electron transfer dissociation (ETD) . Phosphoproteins were generated from protein standard mix including a-casein, b-casein, ovalbumin, BSA, and Ribonuclease B. Our online based system demonstrated that is the powerful tool for analysis of phosphoproteins, which are easy to use and higher recovery of phosphoprotein.

PWe-172
13:30 – 14:40

Label-Free Phosphopeptide Quantitation and Occupancy Determination from a Single Protein Using LC-MALDI

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Keywords:
Occupancy of phosphorylation site, label free quantitation

Novel aspects:
A new approach to quantitatively assign phosphorylation sites was developed combining a dephosphorylation strategy with label-free LC-MS.

Abstract:
Introduction:
Posttranslational modifications in general and protein phosphorylation in particular have gained much attention in proteomics in recent years. Most work focuses on establishing catalogues of phosphopeptides in a proteome of interest and determining whether a certain peptide is phosphorylated or not, but this research stops short of checking for the occupancy of individual residues while verifying multiple phosphorylation sites. It is understood , the degree of phosphorylation at various sites in a single protein can strongly effect its function.
Here, we describe a new approach for the quantitative assessment of phosphorylation occupancy at various previously known target sites within a single isolated protein resembling the situation after immunoprecipitation of a protein in question.

Methods:
The protein KAPCA_HUMAN, containing 10 known phosphorylation sites, was used to quantitatively determine the occupancy of all sites. After EDTA-removal, the sample was trypsinized and split into two aliquots. One aliquot was enzymatically de-phosphorylated in contrast to the other aliquot, so that a native (N) and a dephosphorylated (D) peptide aliquot were obtained. Aliquots were analyzed in replicates using nanoflow LC-MALDI-TOF/TOF for peptide identification and the label-free quantification of the relative abundance of the free, unphosphorylated peptides. The intensity of the D-peptides was assumed to be 100 % free peptide. By subtracting the lower intensity of the N- peptides (1 -x) , the occupancy x [%] could be determined.

Results:
KAPCA_HUMAN (cAMP-dependent protein kinase catalytic subunit alpha) contains 10 phosphorylation sites of which 8 are partially and 2 are stoichiometrically phosphorylated. The protein was obtained in highly purified form from FW Herberg, Kassel. For the determination of the occupancy, 3 replicate LC runs of 1 pmol of each the native (N) and the dephosphorylated (D) tryptic digest were performed. MALDI-MS/MS and Mascot searching provided peptide sequence verification. The native sample yielded pairs of phosphorylated and non-phosphorylated peptides with the same sequence. Unique to this method, only the non-phosphorylated peptides were used for the occupancy determination and the use of LC equipment was not required.
The occupancy of the phosphorylated residues was calculated by (1 - N/ (N+D)) *100 [%] , where N and D represent the abundance of the native and the dephosphorylated peptide, respectively. As expected, the peptides containing the stoichiometrically phosphorylated residues were detected only as phosphopeptides in the N aliquot confirming 100 % phosphorylation. Three other sites were non-stoichiometrically phosphorylated at Ser10 (93 %) , Ser139 (37 %) , and Ser259 (23 %) . For 5 residues, phosphorylation was not detectable, implicating that the degree of phosphorylation at these 5 sites was less than 2 % . The abundance of these non-phosphorylated peptides in the D and N aliquots were equally abundant within the quantification error tolerance. The coefficients of variation (CVs) of the label-free dephosphorylation-LC-MALDI method are within a range of 10 to 30 % in agreement with established and cost intensive isotope labeled reference methods (WD Lehmann) .
An interesting side aspect of this work was finding that trypsin cleavage was impaired near phosphorylation sites, changing the digestion characteristics depending on the modification state. This can effect any peptide based quantitative proteomics strategy and thus discourages quantitative high-throughput shotgun phosphoproteomics work.

PWe-173
11:10 – 12:20

Ion-Trap Quantitative Proteomics of Glycoproteome from Pancreatic Cancer Patients based on the Label-Free and iTRAQ Approaches

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Keywords:
ion trap, glycoproteome, label-free, iTRAQ, pancreatic cancer

Novel aspects:
The obtained results bring insight into quantitative proteomics using ion-trap MS. Described data show differences in the glycoproteomes between pancreatic cancer patients after gemcitabine treatment, indicating responders vs. non-responders.

Abstract:
In order to manage the requirements of current biological and medical studies, designed to understand molecular and cellular mechanisms, the "omics " techniques have evolved into sophisticated and advanced tools. Whereas most of the initial proteomic studies were focused on proteins identification, modern mass spectrometry (MS) technology provides useful platforms to investigate quantitative changes in protein components. Quantitative proteomics can be successfully used in characterizing alterations in protein abundances as a consequence of pathophysiology or progressing treatment of a disease. This is based on an assumption that such differences represent differential protein expression originated from particular conditions of the biological system.

Proteins gained from different samples should be digested by proteolytic enzyme, and separately labeled at their amino groups with one of the Isobaric Tags for Relative and Absolute Quantification (iTRAQ) . The resulting labeled peptides are then combined and fractionated by multidimensional chromatography, and quantitatively analyzed by MS. The intensities of these fragments are used for quantification of individual peptides representing various proteins. Originally, iTRAQ was devoted for measurements by the MALDI TOF/TOF method, followed by other linear quadrupole-based analyzers. Our studies show how to overcome the major obstacle of this method, and enable the use of an ion-trap instrument to minimize the cut-off at the low m/z range.

Another presented approach is based on the label-free technology, which allows for quantitation of peptides using spectral characteristics, such as peak area or frequency of peptide fragment peak during nanoLC-MS/MS analysis, named spectra counting. Peptide peak intensity or spectral counting is measured for individual nanoLC-MS/MS runs, and changes in protein abundances are calculated via direct comparison between subsequent analyses. Spectral counting facilitates faster scan rates, higher intensity, and rapid MS to MS/MS switches, while peak integration benefits from constant retention time gained from the nanoLC system and high accuracy mass analyzers. The data processing pipelines typically include data normalization, retention time integration, peak detection, identification, matching, quantification, and statistical analysis. The main obstacle of this approach is the necessity to gain sufficient reproducibility of the retention times for corresponding peaks.

Presented studies based on quantitative analysis of peptides obtained from standard protein tryptic digest were analyzed with the Ion-Trap Amazon ETD (Bruker Daltonics, Germany) mass spectrometer combined with Proxeon nanoLC system. Nano-LC chromatography used for the experiments consisted of two orthogonal separation techniques, Hydrophilic Interaction Liquid Chromatography (HILIC) and Reversed Phase (RP) chromatography. Such an approach provided an evidence, that an ion-trap instrument is able to perform both iTRAQ and Label-Free based quantitative analysis. Based on this knowledge, we analyzed sera obtained from pancreatic cancer patients after gemcitabine treatment. Quantitative analysis of glycoproteins fraction after Lectin Affinity Chromatography (LAC) was carried out following HILIC and RP nanoLC separations. The obtained peptides were analyzed using MS/MS.

The results suggest, that individual peptides in complex biological systems might be quantified subsequently to sufficient sample separation. However, special concern should be devoted to the selection of the appropriate quantitation approach according to the needs of sample quality and experimental design.

This work was supported by the grant from The Polish Ministry of Science and Higher Education N N403 086039.

PWe-174
13:30 – 14:40

Screening of Oxidative Stress induced LDL-modifications using (LC-) MALDI-MS(/MS) based Lipoproteomics

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Keywords:
Atherosclerosis, Oxidative stress, Lipoprotein modifications, Clinical diagnostics, MALDI-QIT-TOF-MSMS

Novel aspects:
A novel software assisted LC-MALDI-MS/MS based platform with potential for clinical monitoring of lipoprotein modifications is introdced.

Abstract:

Introduction

Oxidative modification of low-density lipoprotein (LDL) induced by reactive oxygen species and/or elevated glucose levels is considered a major risk factor of cardiovascular disease (CVD) and atherosclerosis. Our aim is to identify the modifications of oxidized LDL (OxLDL) directly at the constituent phospholipid (PL) and amino acid (AA) sequence levels (e.g. of the ApoB-100 protein) . For this purpose we introduce a lipoproteomics approach using (LC-) MALDI-MS (/MS) combined with an intelligent software based screening tool.

Methods

Lipoproteins were isolated from normal human plasma by preparative ultracentrifugation and modified by diverse reactive oxygen species and lipid peroxidation products (e.g. MDA, HNE) in the presence or absence of sugars (e.g. glucose) . PLs were extracted and directly analyzed by MALDI-MS. The apolipoproteins were digested with endoproteinases (e.g. trypsin) , peptides were separated by HPLC and sequence mapping was performed based on MALDI-MS/MS. Mass spectra were recorded using MALDI-TOF-RTOF- and MALDI-QIT-TOF-MS (/MS) platforms, respectively. Data were processed and searched for specific MS/MS signatures based on a parameterizable database and software module (PTM-Finder™, Shimadzu) .

Preliminary Data

LDL oxidation increased MDA levels (> 30 nmol/mg protein) and signal intensity of LPCs (e.g. LPC16 : 0) but decreased signal intensity of *sn*-2 PUFA containing PCs (e.g. PAPC, PLPC) indicating for lipid peroxidation and increased activity of lipolytic enzymes (e.g. Lp-PLA$_2$). Moreover, large MS/MS dataset (more than 400 MS/MS spectra per LDL type) were monitored to identify user defined modifications at the AA level. Based on this approach in glycated LDL (gLDL) two distinct glycation sites located within the LDL-R binding domain of ApoB-100 were found. Furthermore, additional sets of MS/MS spectra from other OxLDL samples (e.g. Cu-LDL, HOCl-LDL) were screened for characteristic protein carbonylation products (e.g. Michael adducts) .

PWe-176
13:30 – 14:40

In-depth human proteome analysis by one-shot LC-MS/MS with meter-scale monolithic silica capillary columns

Mio Iwasaki[1], Masaki Wakabayashi[1], Naoyuki Sugiyama[2], Nobuo Tanaka[3], Yasushi Ishihama[1]

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Keywords:
Human proteome / LC-MS/MS / HeLa cell / monolithic silica column

Novel aspects:
This 'one-shot' proteomics using one-dimensional separation by 600 cm monolithic silica column showed a possibility to unveil entire human proteome with small sample amount.

Abstract:
<Introduction>
Shotgun proteomics based on capillary liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been playing important roles for analyzing protein samples. A rapid progress has been made in mass spectrometric technologies such as high sensitivity and high scan speed, resulting in improvement of the identification efficiency of proteome. However, it is still difficult to analyze the entire proteome of mammalian cells.
In shotgun proteomics, protein digests from cells have a variety of peptides with extremely high complexity and the wide concentration range. That is why various kinds of multidimensional separations have been employed for reducing sample complexities and improving identification efficiency of proteome. However, this approach has demerits of increased sample amount and long analysis time.
An alternative approach to improve the identification efficiency in shotgun proteome analysis is to increase the separation efficiency in one-dimensional reversed phase liquid chromatography. Monolithic silica columns offer high-efficiency separation with long column formats because of their high permeability [ref. 1] . We have recently been developing one-dimensional LC-MS/MS systems with meter-scale monolithic silica columns for *E. coli* and human proteome analysis.
With triplicate LC-MS/MS analyses (41 hours gradient) using 350 cm long monolithic silica columns, we identified 2,602 proteins for the *E. coli* cells, in which the equivalent number of genes was detected by DNA microarray analysis [ref. 2] . For human proteome analysis, 5,970 proteins were identified for the HeLa cells by quadruplicate LC-MS/MS analyses (8 hours gradient) using 400 cm long monolithic silica column, which is the best result yet reported without the use of exhaustive pre-fractionation [ref. 3] . Compared to a conventional particle-packed column system, 5-fold improvement in MS responses was observed by the reduced co-elution of peptides using improved separation efficiency of monolithic silica column system for the sample of both species. However, we did not observe dynamic range extension for the identified human peptides as our *E. coli* proteome analyses, suggesting that current analytical technologies are still not adequate for highly complex human peptide mixtures. Our objective is to improve one-dimensional separation efficiency to achieve the identification of entire human proteome with small sample amount. In this study, we used HeLa protein digest and examined one-dimensional LC separation with a 600 cm long monolithic silica column.

<Results and discussion>
Using the 600 cm long monolithic silica column, we evaluated the effect of the gradient time for the analysis of HeLa cell lysates. As expected, the number of identified proteins was remarkably increased as the gradient time became longer. Consequently, 36,701 non-redundant peptides from 7,112 proteins were identified by the single LC-MS/MS analysis of 15 μg sample with 60 hour gradient time. In total, 59,182 non-redundant peptides from 9,500 proteins were identified by the combined LC-MS/MS analyses using 60 μg injected sample and the total analysis time of less than 7 days. Because this system does not need any additional experimental fractionations prior to LC-MS/MS, this 'one-shot' proteomics approach allows simplifying the workflow of shotgun proteomics and minimizing the sample amount as well as the total analysis time even when the shallow gradient was applied.

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PWe-177
11:10 – 12:20

Automated Quality Control of Biopharmaceutical N-/C-Terminal Sequences by Routine Top-Down Mass Spectrometry

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Keywords:
Automated sequence validation in recombinant protein QC, top-down analysis, in-source-decay using MALDI-TOF/TOF, N- and C-terminal sequencing, TDS-MALDI

Novel aspects:
Automated sequence validation of MALDI-TDSdata for routinely assigning the status of N- and C-termini of biopharmaceutical proteins.

Abstract:

Introduction:
During production of recombinant proteins, e.g., for pharmaceutical application, fast and reliable QC methods are needed. Mass spectrometric analysis of tryptic protein digests , also known as "bottom-up " analysis, is typically used for that purpose providing for high sequence coverage of the target protein. However, terminal sequences are not safely covered by this approach. Top-down sequencing (TDS) analysis such as In-Source-Decay (ISD) using MALDI-TOF/TOF instrumentation produces N- and C-terminal fragments covering up to 80 amino acids from either end. Sample preparation, spectrum acquisition and interpretation of the TDS-MALDI data only take minutes to assign N- and C-termini on a routine base.

Methods:
The proteins bovine RNAse B, chicken lysozyme, bovine carbonic anhydrase, BSA and the 116 kDa -galactosidase were analysed by MALDI-TDS following standard procedures. A software method was developed for fast and simple validation of the terminal sequences of isolated proteins with MALDI-Top-Down-Sequencing (MALDI-TDS) using ISD. ISD spectra were matched automatically with the theoretical TDS-spectra calculated from suggested sequence candidates. The quality of the match was scored. The acquired ISD spectrum, the best matching protein sequence, and the detailed N- and C-terminal sequence assignment were assembled as a PDF report for further manual analysis or documentation.

Results:
Protein ISD-spectra were automatically matched against a set of expected protein sequences that also included possible terminal or side chain modifications. The software calculated a, c, y and z+2 ions for each protein sequence and matched the theoretical fragments against the spectrum. A scoring algorithm identified the best match. The results were assembled in an in-depth PDF document that displayed the annotated ISD spectrum together with the protein sequence for the best matching protein in a sequence coverage map. For all other protein sequence candidates the information about a confirmed N- or C-terminus is listed as well. The reports are collected in a common report repository and include assignment of modifications in the range covered by the termini analysis (e. g. residues 1-80 from both sides) .
Using a batch process it was possible to match a series of spectra against a series of protein sequences.

The automatic interpretation of TDS spectra was achieved. Terminal truncations, modifications such as acetylation or pyroglutamylation were detected in various investigated proteins as well as terminal sequence extensions. MALDI-TDS permitted assigning the status of protein termini even in a fully automated fashion.

PWe-178 Evaluation of targeted proteomic approaches on a Q-TOF system

13:30 – 14:40

Carsten Funke, Stepahnie Kasper, Wolfgang Jabs, Carsten Baessmann

Bruker Daltonik GmbH

Keywords:

Targeted proteomics, high mass accuracy, QTOF, quantitiation

Novel aspects:

The targeted quantification efficiency of a Q-TOF system is determined using proteomic standards covering a broad concentration range.

Abstract:

Mass spectrometry-based quantitative proteomics is a popular tool for biomarker discovery. Advances in the speed of separation of proteomics samples and the quality of mass spectrometric detection also enable the subsequent validation of discovered protein biomarkers in complex biological matrices using targeted approaches. These biomarker validation approaches heavily rely on methods exactly targeting and measuring the quantity of specific peptides and proteins in complex proteomic samples. Typically, SRM and MRM (single- and multi-reaction monitoring) approaches are applied for validation tasks. Nevertheless, those methods require detailed target knowledge and do not allow re-examining of acquired data.

We describe here targeted proteomics approaches using the high resolution and accuracy achievable with an Ultrahigh Resolution (UHR) Q-TOF system. Different data acquisition methods are used for targeted quantitative proteomics, namely MS-based acquisition, broad band collision induced dissociation (CID) , and middle band CID.

Target protein quantification is done in a complex *E.coli* background (500ng) spiked with different amounts of a proteomic standard, which spans a concentration range of 6 decades in total (UPS-2, Sigma) . Protein identification information, including m/z values and retention times, used as targets for quantification is obtained out of the equimolar proteomic standard (UPS-1, Sigma) containing the same set of proteins as the dynamic range mixture. Tryptic peptides are separated on a nanoUHPLC system (U3000 RSLCnano, Dionex) and data acquisition is done using an Ultrahigh Resolution (UHR) -TOF (maXis impact, Bruker Daltonics) equipped with a Captive Spray ionization MS source.

Peptide targets for quantitative proteomics are obtained out of an equimolar proteomic standard by a data-dependent MS/MS approach. Results of different identification runs are combined and reveal successful identification of all 48 proteins. Creation of high resolution extracted ion chromatograms used for quantification is done based on the information of identified peptides with regard to m/z values and retention. Quantification of the target peptides spiked into *E.coli* background based on pure MS data acquisition reveals reliable results of targeted proteomics. In total 28 proteins were significantly regulated ($p<0.05$) demonstrating a high quantification efficiency from 500 fmol down to the amolar range. Results of targeted proteomics approaches using broad band and middle band CID will not only provide quantitative but also qualitative data in single runs. Quantification efficiency with regard to the coverage of a high dynamic range of these methods will be compared to the pure MS-based acquisition.

PWe-179
11:10 – 12:20

Electron Transfer Dissociation differentiates the isobaric deamidation products Aspartate and IsoAspartate.

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[1]Bruker Daltonik GmbH, [2]Biocon

Keywords:
ETD, ion trap, isoaspartate

Novel aspects:
ESI-Trap ETD fragmentation provides a simple method of distinguishing asparagine deamidation and aspartate isomerization in biopharmaceutical antibodies

Abstract:
Biopharmaceuticals, in particular monoclonal antibodies, are set to revolutionize healthcare of the future. It is expected that by 2014 five of the top ten best selling pharmaceuticals will be derived from proteins or peptides. Degradation of these protein drugs results in reduced potency and increased immunogenicity. Deamidation of asparagine is a major cause of protein degradation and to protein folding disorders such as Alzheimer's disease. Aspartic acid formed by nonenzymatic deamidation of asparagines often isomerizes to isoaspartic acid. Accumulation of isoaspartic acid initiates protein aggregation and protein degradation. With CID fragmentation it is impossible to discriminate aspartate from isoaspartate residues. However, ETD fragmentation produces a signature backbone cleavage which can be used to differentiate Asp and IsoAsp containing peptides.
Monoclonal antibodies were incubated in 4 M guanidium chloride, with 50 mM Tris-HCl and 0.5M EDTA, ph 8, and reduced with 10 mM dithothreitol for 15 min, followed by carbamidomethylation with 20 mM iodoacetamide for 30 min. The resultant heavy and light chains were separated and purified via size exclusion chromatography (TSK-GEL G400SW column, Tosoh Bioscience) in preparation for protease digestion. The tryptic peptides were separated using a C18 (4.6 mm_250 mm, Grace-Vydac) reverse-phased column and injected online to an ESI ion trap mass spectrometer (HCTultra ETD, Bruker Daltonics) . For collision-induced dissociation (CID) the trap was operated in autoMS/MS (UltraScan) mode. For ETD low-energy electrons were generated by electron impact of methane and transferred to fluoranthene to generate negatively charged fluoranthene ions.
Tryptic peptides were separated on a reversed-phase C18 column and detected online through ESI-MS/MS. The tryptic peptides were identified from the MS profile. The peptides containing asparagine were specifically investigated for a change in mass and retention time. An increase in mass of 1 Da was detected in two of the asparagine-containing peptides from the heavy chain constant region. The reversed-phase HPLC method was able to separate three related species from both Asn containing peptides, these were the original Asn containing peptides and the suspected Asp and IsoAsp containing peptides. Examination of the b and y type ions from the CID MSMS spectra of the suspected Asp and Iso Asp peptides conclusively confirmed the inclusion of an increased mass of 1 Da which enabled identification of the site of deamidation. However, due to the isobaric nature of the peptides, CID fragmentation products from the Asp and IsoAsp containing peptides were found to be identical and therefore CID was unable to differentiate between the aspartate isomers.
Formation of isoaspartate results in an extra methylene unit in the peptide backbone. Under ETD fragmentation, identical c and z type ions were found in the spectra of Asp and IsoAsp peptides. However, the isoaspartyl peptides also displayed a unique fragmentation signature (C_n + 58 Da and z_{L-1} - 57 Da) which was not observed in the Asp peptides. These results demonstrate that ETD fragmentation is a convenient method for monitoring and differentiating site-specific asparagine deamidation and aspartate isomerization in a single MSMS experiment performed during routine peptide mapping of biopharmaceutical antibodies.

PWe-180
13:30 – 14:40

Identification and quantification of concentration-dependent biomarkers in MCF-7/BOS cells exposed to 17beta-estradiol by 2-D DIGE and label-free proteomics

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Keywords:
Biomarkers, estrogens, food-chain security, proteomics, MCF-7/BOS

Novel aspects:
2D-DIGE and label free proteomics coupled with PCA analysis allowed the discovery of estrogen activity biomarkers that can be used for rapid screening of estrogenic activity of xenobiotics.

Abstract:
The rapid screening of xenoestrogens prior to heavy analytical techniques is an important step in environmental pollution monitoring and food chain safety.
We report the identification of biomarkers resulting from the exposure of MCF-7/BOS cells to 17beta-estradiol (E_2). The biomarkers were identified from the cytosolic fractions of cells treated for 24 h with mitogenic concentrations of 1, 30 and 500 pM of 17beta-estradiol.The biomarkers were identified using 2 independent and complementary techniques, 2-D DIGE / MALDI-TOF peptide mass fingerprint, and 2-D UPLC-ESI MS/MS. To enable the further addition of new chemicals in the study, the 2D-DIGE experiment were performed using, instead of the classical internal standard, a reference gel. A robust PCA analysis allowed to filter the biological variability and recover a dose dependence variation. Five biomarkers were up-regulated proteins, HSP 74, EF2, FKBP4, EF 1 and GDIB and one was a down-regulated protein, K2C8. Three of these proteins, EF2, FKBP 4 and K2C8 are implicated in a network centred on the estrogen receptors ESR 1 and ESR 2 as well as on AKT1. After the discovery phase, three biomarkers were selected as a signature for the response to the presence of estrogens. They were monitored using SRM after incubation of MCF-7/BOS in the presence of E_2 for confirmation or selected xenoestrogens. Daidzein, coumestrol and enterolactone induced an up-regulation of EF 2 and FKPB 4 proteins, while tamoxifen and resveratrol induced a down-regulation. The exposure of all phytoestrogens induced the down-regulation of K2C8. These markers form a preliminary molecular signature that can be used when testing the estrogenic activity of new chemicals, xenobiotics, either pure or in mixtures extracted from food chain or environmental samples.

PWe-181
11:10 – 12:20

Quantitative phosphoproteome analysis of apoptotic human cell cultures using SILAC combined with complementary ESI-MS techniques

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Keywords:
Quantification of phosphoproteome, apoptosis, SILAC, combined approach using of ETC on ion trap and ESI-UHR-TOF

Novel aspects:
First quantitative MS-analysis of apoptosis-related phosphoproteome and first application of complementary ESI-MS techniques for the quantitative analysis of phosphopeptides.

Abstract:
Introduction

Apoptosis, the main form of programmed cell death, plays an important role in multicellular organisms as it is involved in modeling of tissue structures, elimination of mutated and damaged cells and homeostasis. Hence, a dysfunction can lead to severe diseases, such as cancer, autoimmune diseases or neurodegenerative disorders. Furthermore, in bioreactor cultivations of animal cells apoptosis negatively affects the production of biopharmaceuticals.
As protein phosphorylations are of great importance for apoptosis regulation and induction, the elucidation of changes in the phosphoproteome would lead to a better understanding of apoptosis-related diseases and new solutions for handling animal cells in bioreactor cultivations. Here, we present a comprehensive approach combining SILAC and complementary ESI-MS techniques for identification and quantification of apoptosis-involved protein phosphorylations.

Methods
Cultivation of Jurkat ACC 282 cells was carried out in shaking flasks in chemically-defined, serum-free medium. Three of six replicates were grown in heavy medium, containing stable isotope labeled lysine and arginine, and induced for apoptosis with 100 uM etoposide while the other three replicates grown in light medium were left untreated. 4 h after apoptosis induction cells from treated and untreated cultures were harvested and mixed followed by protein extraction. 3 mg of total protein extract were separated by SDS-PAGE. The gel was split into 10 horizontal strips. Each strip was cut out, in-gel digested with trypsin and enriched for phosphopeptides via IMAC. The samples were analyzed by nanoLC coupled to an ESI (CID/ETD) -ion trap and an ESI-UHR-TOF, respectively.

Results
Previous experiments of apoptosis induction in Jurkat cells showed that 100 uM etoposide led to activation of the apoptotic execution phase after 5 hours. In order to see changes of the phosphoproteome during the induction phase of apoptosis a sample point of 4 hours after etoposide treatment was chosen. At this time point, comparing treated and untreated cells no difference in viability and viable cell density was observed. XX h after induction clear signs of apoptosis were observed. While the untreated cells continued to growth to a viable cell density of XXXXX the treated cultures dropped to a VCD of XXX and a viability of XXX (9X % in untreated cells).
Our method gives access to more than 2000 quantifiable phosphopeptides, including numerous apoptosis-related proteins. The use of complementary MS-techniques combines the advantage of ETD and CID fragmentation supplied by the utilized ESI-ion trap with the strength in quantification offered by an ESI-ultra high resolution-TOF. In test runs analyzing complex samples we could show an increase of about 50% in phosphopeptide identification by the additional use of ETD-fragmentation.
The elucidation of changes in the phosphoproteome of apoptotic human cell cultures will lead to new strategies for the suppression of apoptosis in production processes of biopharmaceuticals. Since phosphorylations are involved in early events of apoptosis induction, manipulations focusing on apoptosis-related phosphorylations would lead to a more effective inhibition of apoptosis.

PWe-182
13:30 – 14:40

Validating a novel Parkinson's Disease SRM-based assay using automated sample preparation

Christine A Jelinek[1], Liana Rosenthal[1], Yan Jia[1], Kevin W Meyer[2], Ted Dawson[1], Robert J Cotter[1]

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Keywords:
post-translational modifications, parkinson's disease, selected reaction monitoring, mass spectrometric assay, biomarker

Novel aspects:
automating front-end protoemic sample preparation for an SRM-based novel Parkinson's Disease diagnostic assay increases throughput but decreases assay analysis time and experimental variability

Abstract:
Parkinson's Disease is the second most common neurodegerative disorder world-wide. Given that early intervention is associated with improved quality of life and lifespan for afflicted patients, it is especially unfornate that currently there is no biomarker or diagnostic test available for Parkinson's Disease (PD) . To address the need for a PD diagnostic, we have developed a selected reaction monitoring (SRM) assay to monitor alpha-synuclein post-trannslational modificaitons associated with PD pathophysiology. Using Selected Reaction Monitoring-mass spectrometry, six phosphorylated, two nitrated alpha-synuclein peptides, and their corresponding tryptic peptides will be measured in cerebrospinal fluid of PD patients with and without cognitive impairment and healthy controls. Our screen will help determine if modified alpha-synuclein contributes to the pathophysiology of PD and/or if the selected alpha-synuclein peptides could serve as clinical diagnostics. Unlike assays that require multiple manual sample preparation steps, the sample preparation for our developed assay occurs with the Perfinity Workstation. Replacing manual preparative steps with column-mediated digestion, desalting, and Reverse Phase-HPLC separation has greatly improved the reproducibility and throughput of our SRM-assay--critically important features for a clinical assay.
The SRM platform used during assay development and validation consists of a Perfinity Workstation (Perfinity Biosciences, Indiana) placed on-line to a triple quadrapole mass spectrometer. The SRM-software Skyline directed iterative assay development. To facilitate quantitation of the sixteen alpha-synuclein peptides, three signature apomyoglobin peptide standards have been added to samples as external standards. The sixteen alpha-synuclein peptides have been defined by a minimum of three transitionions. Selected transitions were chosen both for uniqueness and relative intensity. The assay protocol is as follows：using the Perfinity workstation, 200 microliters of individual CSF obtained during lumbar puncture was diluted 1 ： 5 in .1% Rapidgest, 50 microliters of which was then injected, digested on-column in six minutes, desalted on-column, and then subjected to 30 minute LC-SRM analysis.

Using SRM-MS placed in-line with a Perfinity Workstation to construct a PD-diagnostic assay, we have successfully screened 10 control subjects using our workflow. As anticipated, SRM-MS displays the sensitivity and specificity needed to detect low abundant candidate biomarkers in complexbiological fluid like CSF. For each of the twenty CSF samples we have screened using the developed assay (ten patient samples acquired in duplicate) , all nineteen monitored peptides were detected in sufficient abundance so as to facilitate downstream quantitation. Furthermore, replacing traditional bench-top sample preparation with the Perfinity Workstation has ensured that the technique is both high through-put and highly reproducible. Using the Perfinity Workstation for our sample preparation ensures analysis is completed in under 45 minutes for each patient sample. Analysis time now includes all sample preparation required for SRM-based mass spectrometry (even tryptic digestion) . Additionally, for our ten patient control subject cohort, our assay generated %cvs for all peptides of under10%. Analysis of the six minute tryptic digestion using the uv-vis detector of the Perfinity HPLC suggests that tryptic digestion efficiency for CSF is approximately 90%. For the work here, our developed SRM-MS assay will be used to screen a small clinically relevant PD-patient cohort. The intended cohort will consist of five Parkinson's patients without dementia, five Parkinson's patients with dementia, and five healthy control subjects allrandomly selected for screening in triplicate. Area under the curve measurements of the most intense transition/peptide will facilitate the quantitative approach taken.

PWe-183
11:10 – 12:20

In vitro phosphorylation to manipulate the abundance profiles in proteomic samples

Shunsuke Takagi[1], Haruna Imamura[1], Masaki Wakabayashi[1], Naoyuki Sugiyama[2], Yasushi Ishihama[1]
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Keywords:
LC-MS/MS, phosphoproteomics, in vitro phosphorylation, Low abundant protein, in-depth proteome analysis

Novel aspects:
Manipulate the proteome abundance in the sample by using in vitro kinase reaction followed by phosphoproteomics, in order to achieve the in-depth proteome analysis.

Abstract:
Shotgun proteomics based on LC-MS/MS has been widely used for analyzing proteome profiles in cells of interest. Currently thousands of proteins can be easily identified from single LC-MS/MS run, even when highly complex samples such as cell lysates are analyzed. However, it is still problematic to identify very low abundant proteins in the presence of high abundant proteins. In order to solve this problem, pre-fractionation approaches prior to LC-MS/MS have been employed. However, we cannot avoid the trade-off between the higher coverage by multidimensional separation and the longer analysis time by the increased number of fractions.

Here we introduced a novel approach to manipulate the proteome abundance in the sample by using *in vitro* kinase reaction followed by phosphoproteomics.

HeLa cells were dissolved in 12 mM sodium deoxycholate and 12 mM sodium lauroylsarcosinate, followed by trypsin digestion [1]. The digested peptides were treated with phosphatase to remove the endogenous phosphorylations. The dephosphorylated peptides were phosphorylated by spiked kinases *in vitro*. Finally, the phosphorylated peptides were enriched by HAMMOC (hydroxy acid-modified metal oxide chromatography) with titania [2] and analyzed by nanoLC-MS/MS using ABSciex TripleTOF 5600. The MS was equipped with a Dionex UltiMate 3000 RSLCnano pump and an HTC-PAL autosampler. LC column was packed in house with ReproSil-Pur C18-AQ (3 μm particle size, 120 A pore size, 100 μm i.d., 15 cm long). The injection volume was 5 μL, and the flow rate was 500 nL/min. The mobile phases consisted of (A) 0.5 % acetic acid and (B) 0.5 % acetic acid and 80 % acetonitrile. A three-step linear gradient of 5-10% B in 5 min, 10-40% B in 60 min, 40-100% B in 5 min, and 100% B for 10 min was employed for the peptide elution.

Since each kinase has phosphorylation motifs to the substrate sequence, it phosphorylates proteins or digested peptides selectively, and we can extract the substrate proteins/peptides by using the phosphoryl group as the target of chemoaffinity purification based on phosphoryl group-metal oxide interaction. Consequently, peptides with the motif sequence are enriched and the rest of peptides are discarded. Thus, the complexity in samples is drastically reduced, enabling to identify low abundant proteins more efficiently.

In this study, 4 Ser/Thr kinases (Ck 1 d, Ck 2 a2, Erk1, Erk 2) and 2 Tyr kinases (EPHB1, JAK 2) were employed. Each kinase was reacted with 100 μg of HeLa cell digests and triplicate analyses were done. As a result, we identified 2542 non-redundant proteins. Among them, 310 proteins were not identified by the conventional approach [ref 3] (4634 proteins were identified). Because Tyr kinases are generally less selective, more proteins were identified (754 and 2287 proteins from Ser/Thr kinases and Tyr kinases, respectively). On the other hand, the percentage of Ser/Thr kinase-derived proteins identified exclusively by this approach was higher than that of Tyr kinase-derived proteins. Although further optimization is required to increase the substrate proteins, this approach would be useful to manipulate the proteome profile of the samples to achieve the in-depth proteome analysis.

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PWe-184
13:30 – 14:40

Identification of kinase substrates by siRNA-induced kinase knock-down coupled with quantitative phosphoproteomics

Ayaka Sato[1], Sayaka Iwano[1], Wei-Chi Ku[1], Masaki Wakabayashi[1], Naoyuki Sugiyama[2], Fumiko Toyoshima[1], Yasushi Ishihama[1]

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Keywords:
phosphoproteomics / LC-MS/MS / siRNA / kinase knockdown

Novel aspects:
Kinase substrates are identified with siRNA and quantitative phosphoproteomics.

Abstract:
Protein phosphorylation, one of the most ubiquitous post-translational modifications, is a key module in protein kinase-mediated cellular signal transduction, regulating a wide range of cell functions. It has been extensively identified by shotgun phosphoproteomics with highly selective phosphopeptide enrichment. However, for most of the phosphorylation sites, we do not know which kinase is responsible *in vivo*. Therefore, we developed a novel approach to identify kinase substrates by knocking down the kinase followed by phosphoproteomic analysis to quantify the phosphorylation level of substrate candidates. As a model case, we employed serine threonine protein kinase PCTAIRE-1 (PCTK1) to be knocked down by siRNA, and quantitative phosphoproteome analysis was carried out.

HeLa cells with or without PCTK1 siRNA were prepared. After lysing the cells, proteins were extracted and digested by trypsin after reductive alkylation. The resultant sample was desalted using reversed-phase StageTips with C18 disk membranes. Phosphopeptide enrichment was carried out by hydroxy acid-modified metal oxide chromatography (HAMMOC) with titania. An AB SCIEX TripleTOF 5600 system (Foster City, CA, USA) equipped with a Dionex UltiMate 3000 RSLCnano pump and an HTC-PAL autosampler (CTC Analytics, Zwingen, Switzerland) was employed for phosphopeptide analyses. ReproSil-Pur 120 C18-AQ materials (3 mm, Dr. Maisch, Ammerbuch, Germany) were packed into a self-pulled needle (150 mm length_100 mm i.d., 6 mm opening) to prepare an analytical column needle with 'stone-arch' frit. The injection volume was 5 mL and the flow rate was 500 nL/min. Mobile phases were (A) 0.5% acetic acid in water and (B) a mixture of acetic acid (0.5%), water (19.5%) and ACN (80%). The gradient condition was employed : 5% to 10% B in 5 min, 10% to 40% B in 60 min, 40% to 100% B in 5 min and 100% B for 10 min. A spray voltage of 2400V was applied. The MS scan range was m/z 350-1400. The top ten precursor ions were selected for subsequent MS/MS scans.

At first, in order to investigate the influence of PCTK1 on the signal transduction pathways, we prepared HeLa cells treated with siRNA of PCTK1, confirming clear phenotypes. Then cells with overexpressed PCTK1 as well as their control cells were additionally prepared. These four samples were applied to quantify phosphoproteomics at label-free quantitation mode. As a result, 19 phosphopeptides were selected out as the downstream molecules regulated by PCTK1 according to the quantitation criteria in which the phosphorylation level was decreased in the siRNA samples, whereas increased in PCTK1-overexpressed samples. To specify the direct substrate molecules of PCTK1, we performed *in vitro* kinase assay using recombinant PCTK1 and HeLa cell extracts. We successfully identified 81 phosphopeptides from 63 proteins. Motif extraction was carried out by Motif-X algorithm, providing [.....SPP...] . By matching this motif with the 19 phosphopeptides, two candidates, cAMP-dependent protein kinase type 1-alpha regulatory subunit (KAP0) and heat shock factor protein 1 (HSF1), were selected. Especially, the former phosphopeptide was also identified as the *in vitro* substrate. It was found that the kinase knock-down together with *in vitro* kinase assay and quantitative phosphoproteomics was a powerful tool to determine the *in vivo* kinase substrates. Since this approach can be extended to larger-scale analysis, it will help to give more information about cellular phosphorylation network.

PWe-185
11:10 – 12:20

Comprehensive Targeted Quantitative Proteomics - Assessing Quantitation Quality of Highly Multiplexed Assays

Christie L Hunter, Sean L Seymour

AB SCIEX, Foster City, CA, United States

Keywords:
SWATH acquisition, quantitative proteomics, data independent acquisition

Novel aspects:
Comprehensive quantitative proteomics analysis via data independent SWATH acquisition

Abstract:
The goal of quantitative proteomics is to both identify and quantify a broad range of proteins and peptides. The extreme complexity and dynamic range of proteins in typical proteomic samples challenges traditional data dependent workflows by requiring very high speed MS/MS acquisition to reproducibly and deeply interrogate the sample. The application of data independent acquisition strategies to increase the reproducibility and comprehensiveness of data collection has been limited by the speed of current mass spectrometers. Recent QqTOF innovations providing high speed acquisition of high resolution MS/MS spectra have enabled a new data independent acquisition strategy. In this workflow (called MS/MS^{ALL} with SWATH™ Acquisition) , the Q1 quadrupole is stepped at 25 amu increments across the mass range of interest, passing a 25 amu window through into the collision cell. The transmitted ions are fragmented and the resulting fragments are analyzed in the TOF MS Analyzer at high resolution. This is done in a looped fashion with an LC compatible cycle time, such that MS/MS spectra has been acquired on every peptide in the sample. Because the fragment ions are collected at high resolution, high quality XICs can be generated post-acquisition to produce the MRM-like data.

The utility of this workflow for highly multiplexed targeted quantification in complex proteome samples will be explored. Working from peptide MS/MS spectral libraries, XICs for target proteins and peptides can be generated from this comprehensive dataset. Initially, XICs to large #s of peptides and proteins were extracted and analyzed for quantitative reproducibility. Impact of various acquisition conditions on quantitative specificity was explored and optimized. Finally, this technique will be compared to more common quantitative techniques such as MRM quantification.

PWe-186
13:30 – 14:40

Global profiling and quantification of histones, histone PTM's and histone-modifying enzymes in mesenchymal stem cells using a multiplexed SRM assay

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Keywords:
SRM assay, PTM, Profiling, quantification

Novel aspects:
Development of a multiplexed SRM assay combining histones, specific histone PTM's and histone modification enzymes

Abstract:
Introduction
Histone post translational modifications (PTM's) are a central theme in the regulation of gene expression. A rapidly growing list of modifications confirms that they play fundamental roles in chromatin modeling processes. These processes are also thought to play a role in stem cell development and senescence. To date, most studies in this area have been carried out by genomic analysis, immunostaining, or top-down LC-MS/MS analysis, and as such are not fully quantitative. We have developed a workflow for quantitative global profiling and targeted analysis of histones, histone PTM's and histone modification enzymes using a multiplexed SRM assay.

Methods
Samples
Mesenchymal stem cells were derived from human adipose tissue. Sample lysates were prepared from cell cultures at different ages (Self Renewing (SR) and Senescing (S)) .

SRM assay development and analysis
SRM assays were developed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific) , as previously described. There were three technical replicates per sample, ie each sample was digested once and analyzed three times in the SRM assay. Data were analyzed with Pinpoint™ and Ingenuity pathways analysis (IPA) .

Abstract
A multiplexed SRM assay for ten histone modification proteins and histone H 3 and gamma H 2 Ax sequences including PTM's identified in the literature was constructed. The assay was used to interrogate the S and SR mesenchymal stem cell extracts. This approach was extremely fruitful and allowed us to confirm and quantify previously identified PTM's occurring on histone H 3 and gamma H 2 Ax as well as differential expression of histone-modifying proteins that have not been previously reported in conjunction with either somatic or stem cell aging (replicative or genotoxic stress-induced senescence) .

PWe-187
11:10 – 12:20

Polyhedral approach to comprehensive quantitative analysis for membrane proteomics

Kazuaki Takafuji, Seiji Takashima, Akira Kikuchi

Osaka University, Osaka, Japan

Keywords:
Membrane proteomics Quantitative analysis SRM/MRM SIM

Novel aspects:
Polyhedral approach to comprehensive quantitative analysis

Abstract:
Proteomics analysis is one of the best methods available to study proteins comprehensively.
Recent progress allows to identify thousands of proteins all at once and even to quantitate the amounts of them. However, polytopic membrane proteins that possess several membrane spanning domains, such as transporters, have been omitted and/or ignored by most of comprehensive proteomics analysis, because these proteins are very difficult to handle due to their hydrophobic properties. Only few researches have focused on quantitation of transporters. Therefore, we have developed the comprehensive quantitative proteomics approach for transporters in mammalian epithelial cells. Brush boarder membranevesicles (BBMVs) were isolated from mouse kidney cortex. Membrane proteins were extracted from BBMVs and digested. The digested peptides were fractionated by chromatography and analyzed by Q-TOF mass spectrometers. Over one hundred transporters were identified and able to be quantitated. Thus, this procedure was performed on BBMVs and total membranes prepared from wild-type and diabetes model mice to compare expression levels of transporters. Alteration of the transporter-proteome were observed in the diabetes model mice, include sugar or drug transporters. This result indicates that the comprehensive quantitative proteomics analysis became a useful tool to study polytopic membrane proteins.

PWe-188
13:30 – 14:40

Separation of polypeptides by isoelectric point focusing in electrospray-friendly solution using multiple-junction capillary fractionator

Konstantin Chingin[1], Juan Astorga-Wells[1,2], Mohammad Pirmoradian Najafabadi[1,2], Thorleif Lavold[2], Roman A Zubarev[1,3]

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Keywords:
capillary isoelectric focusing, polypeptides, online proteomics, mass spectrometry, multidimensional analyses

Novel aspects:
OMJ-CIEF allows solution-phase separation by pI of both proteins and peptides, easy automated online sampling to MS, LC, CE and other capillary-based analyses, and selective mobilization of focused fractions.

Abstract:
The growing popularity of recently introduced IPG-IEF fractionators (e.g., OFFGEL™ fractionator from Agilent) reflects the steady demand for carrying separation of biological samples in solution phase. Direct analyte recovery from solution precludes the pitfalls associated with gel methods, such as tedious and error-prone protocols for sample extraction and purification.
We present an online multiple-junction capillary isoelectric focusing fractionator (OMJ-CIEF) for the separation of biological molecules in solution by pI. Owing to the capillary format of operation, OMJ-CIEF combines the merits of solution-phase separation with the possibility of automated online sampling to MS, LC, CE and other capillary-based analyses.
In OMJ-CIEF, the separation capillary is divided into seven equal sections joined with each other via tubular Nafion membrane insertions. Each junction is communicated with its own external electrolytic buffer which is used both to supply electrical contact and for solvent exchange. The performance of the fractionator was explored using protein and peptide samples covering broad pI range (proteins : β-lactoglobulin A (pI 5.3) , hemoglobin A (pI 6.8) , myoglobin (pI 6.9-7.3) , α-chymotrypsinogen A (pI 9) , ribonuclease A (pI 9.5) , cytochrome C (pI 10-10.5) and lysozyme (pI 11.3) ; peptides : tryptic digest of bovine serum albumin) . Separation was carried in ionic and ampholytic buffers, including ammonium formate, ammonium hydroxide, histidine and arginine. By maintaining electric potential across upstream segments of the capillary after the focusing stage, selective release of downstream analyte fractions could be achieved. The selective release mode circumvents the problem of peak broadening during mobilization and enables convenient comprehensive sampling for orthogonal separation methods. Using single-component ampholyte buffers with well-defined pI cut-off values, controlled separation of protein mixture into basic and acidic fractions was demonstrated.
The device is cheap and easy to fabricate in-house, simple in operation and straightforward in interfacing to hyphened analytical platforms. OMJ-CIEF has a potential of becoming a practical add-on unit in a wide range of bioanalytical set-ups, in particular as a first-dimension separation in mass spectrometry based proteomics or as a preparative tool for analyte purification, fractionation and pre-concentration.

PWe-189
11:10 – 12:20

Comprehensive variability analysis of nutrients absorption mechanism in small intestine by quantitative proteomics

Takashi Nishiyama, Shushi Nagamori, kazuaki Takafuji, Yoshikatsu Kanai

Osaka University Graduate School of Medicine, Suita, Japan

Keywords:
proteomics, membrane protein, transporter, nutrient absorption, sucrose

Novel aspects:
Overall alterations of membrane protein expression by nutrients were revealed by proteomics method.

Abstract:
Introduction

Small intestine is an important organ for absorption of nutrients ; carbohydrate, amino acids, fat, minerals etc. In guts, nutrients are digested by metabolic enzymes and absorbed by transporters. For example, sucrose is hydrolyzed to glucose and fructose by sucrase and absorbed by glucose or fructose transporters. Proteins are digested into peptides and amino acids by peptidases and absorbed by peptide or amino acid transporters. These proteins are express at the epithelial cells, especially brush border membranes of small intestine. There is much evidence that the transporters are adaptively regulated by dietary levels of nutrients. It is important to elucidate the regulation of nutrient absorption for understanding of physiological role or pathology variation of small intestine.

To study the overview of mechanism for nutrient absorption, comprehensive expression analysis of membrane proteins is critical. However, it is difficult to obtain comprehensive data of the expressions by conventional analytical methods, such as western blot or microarray. Microarray is able to evaluate change of mRNA level comprehensively, yet correlation between expression levels of mRNA and protein are not confirmed in many cases. Western blot is one of the most powerful tools to investigate alterations of a protein expression level unless no antibody exists though it is practically impossible to prepare antibodies covers most of proteins.

Proteomics analysis is the best methods available to study proteins comprehensively. Recent progress allows to identify many proteins and even to quantitate the amounts of them. However, polytopic membrane proteins that possess several membrane spanning domains, such as transporters, have been omitted from most of proteomics analysis because of difficulties for handling due to their hydrophobic properties and relatively low level of the expression.

Therefore, we applied a quantitative proteomics approach developed specially for membrane proteins to evaluate the comprehensive variability of proteins in brush boarder membranes of small intestine. Here, we analyzed membrane proteins of intestines from the 3 groups of mice, sugar-drinking, artificial sweetener-drinking and water-drinking (control) groups for a model study.

Method and Results

Mice were separated into 3 groups (sucrose group, sweetener group and control group) , and bred for 2 weeks under free taking of low-carbohydrate diet and each test solutions. There were no significant differences of energy intake and body weight among 3 groups. After 2 weeks, epithelial cells of small intestine (duodenum and jejunum) were isolated by scraping. Brush border membrane vesicles (BBMV) were prepared by divalent cation method for the epithelial cells. Soluble proteins were washed out from BBMV, and then membrane proteins were extracted from phospholipid bilayer membrane by using phase transfer surfactant (PTS) method developed by Masuda et al specifically for extraction of membrane proteins. After the PTS treatment, peptides were fractionated by two-dimensional reverse-phase
chromatography and desalted. The samples were analyzed by MSE method with Q-ToF type LC-MS (Waters, SYNAPT G2 HDMS) .

As the result, a total of 200 proteins were identified and 150 proteins were successful to quantitative analysis among 3 groups. In the sucrose groups, there were marked alterations of expression levels of sugar transporters and metabolic enzymes as reported previously by using the conventional methods. In addition, expression levels of cholesterol transporters and amino acids transporters were also changed. In the sweetener group, no significant alteration of sugar transporters expression level was observed, but there were alterations of expression level of transporters or enzymes that are in charge of amino acids and peptides absorption and metabolism. Taken all together, over all alterations of membrane protein expression by nutrients were revealed by the proteomics method. This method can be use not only for nutrient metabolism but also use for many biological systems which contain membrane proteins.

PWe-190

13:30 – 14:40

Laser Spectroscopic Investigations of 1,3-Dichloro-2-fluorobenzene by REMPI and MATI Spectroscopy

Sascha Krüger, Juergen Grotemeyer

Kiel University, Kiel, Germany

Keywords:

Ion spectroscopy, vibronic structure, excitation/ionization-energy

Novel aspects:

Geometry distortion during ionization

Abstract:

Introduction Ion spectroscopy is an essential method for investigation of ionic molecular species and their vibronic structure. Substitution of one or more hydrogen atoms in the benzene ring leads to significant change in electron density distribution. Previous research showed that certain halogen substitution pattern of benzene lead to strong activity of formally forbidden modes due to vibronic coupling [1] . The aim of this study was to assess the impact of replacing the doubly vicinal chlorine atom in 1,2,3-trichlorbenzene by flourine. The vibronic structure in the first excited state (S_1) was investigated by means of Resonce Enhanced Multi Photon Ionization (REMPI) spectroscopy, the ionic ground state (D_0) by Mass Analyzed Threshold Ionization (MATI) spectroscopy. The experimental findings are supported by quantum chemical calculations.

Experimental The experiment consists of a time-of-flight mass spectrometer equipped with single-stage ion source and reflectron and two tunable dye lasers. The fundamental (1064 nm) of a Nd : YAG pump laser is frequency-doubled to 532 nm. Then, the 532 nm wavelength is mixed with the fundamental to frequency-tripled 355 nm. One dye laser is pumped by the second harmonic of the YAG, the other by the third harmonic. The dye wavelength of the 355 nm pumped laser is frequency doubled in a BBO-I. The second laser was modified to sumfrequency mix the dye wavelength and the 533 nm pump wavelength in a BBO-I. A supersonic molecular beam of sample molecules and seed gas (Argon) is expanded via a pulsed jet valve into the ion source. Excitation or ionization is accomplished by multi photon absorption under field-free conditions. In MATI modus the prompt ions are discriminated against Rydberg neutrals by application of a weak electrical retarding field subsequent to two-photon excitation. Finally, a high voltage puls ionizes the Rydberg neutrals and accelerates them into the TOF. In REMPI modus the retarding field is omitted and the generated ions are accelerated directly into the TOF. A MCP detector is used to detect the ions. The ion signal is readout by a digital oscilloscope.

Results and Discussion The findings from the research provided very accurate values for the electronic excitation (*IE*) and ionization energy (*AE*) of 1,3-dichloro- 2 -fluorobenzene. Furthermore a detailed vibrational analysis could be conducted for the first time. This analysis advances the understanding of vibrational modes active during excitation/ionization. During excitation/ ionization molecules are able to undergo geometric distortion along the eigenvector of certain vibrational modes. The REMPI spectra show unusual high activity of a symmetry forbidden out-of-plane mode ($17b^2$) at low frequencies. Several cases of violation of $v = 0$ propensity rule [2] were observed in the MATI spectra. For instance, a short, three membered progression of the 17b mode with a shift of the Franck-Condon maxmimum to lower energies was observed in the MATI spectrum via $17b^2$. While the calculations suggest a planar molecular structure in the electronic and ionic ground state, they suggest a non-planar structure in the first excited state. The experimental findings and calculation indicate a geometrical distortion during excitation and ionization.

Literature [1] F. Witte, M. Riese, F. Gunzer, J. Grotemeyer, Int. J. Mass Spectrom. 2010, 306 , 129 ; [2] M. Child, Theory of Molecular Rydberg States, Cambridge Molecular Science, Cambridge University Press, 2011.

Poster Session

Wedensday, 19th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PWe-191
11:10 – 12:20

Selective Extraction and Absolute Quantification of Glutathione using Nanoengineered Micro Gold Shells and LDI-TOF MS

Jeongwook Lee, Woon-Seok Yeo
Konkuk University, Korea

Keywords:
glutathione, internal standard, LDI-TOF, quantification

Novel aspects:
Selective extraction and absolute quantification of GSH using organic matrix free LDI-TOF MS with nanostructure embedded gold micro shells.

Abstract:
Glutathione (GSH) is the most abundant biothiol which plays key roles in a variety of biological processes including maintenance of intracellular redox activities, xenobiotic metabolism, intracellular signal transduction, gene regulation, and protecting against oxidative stress. Therefore, the various detection techniques, such as gas chromatography, high performance liquid chromatography, capillary electrophoresis, and optical detection with fluorescence, have been developed for detection of GSH in biological samples.

In this poster, we describe a new method for selective extraction and absolute quantification of GSH using organic matrix free LDI-TOF MS with nanostructure embedded gold micro shells (μAuSs). Our strategy utilizes the Au-thiol interaction which allows the selective extraction of GSH in samples to μAuSs. After the extraction step, the μAuS-bound GSH is analyzed directly by LDI-TOF MS without a use of organic matrix. In addition, GSH in the sample was quantitated by using an internal standard (IS) which was prepared with μAuSs and isotope-labeled GSH. The IS, thus, has the same structure of the GSH but heavy molecular weight and therefore, allowed absolute quantification of GSH by comparing the mass intensities between the GSH and the isotope-labeled GSH. We believe this strategy for absolute quantification of GSH would be useful by providing the quantitative information for diagnostic purposes.

PWe-192
13:30 – 14:40

Coulomb-Interaction-Induced Effects on Xe Isotopic Ion FT-ICR Mass Spectra: A Many-Particle Simulation Using GRAPE

Makoto Fujiwara, Naohisa Happo, Koichi Tanaka

Hiroshima City University, Hiroshima, Japan

Keywords:
FT-ICR, Many-Particle Simulation, Coulomb Interaction

Novel aspects:
Coulomb-Interaction-Induced Effects on Xe Isotopic Ion FT-ICR Mass Spectra were simulated in detail using a more powerful special-purpose computer for many-body problems, GRAPE

Abstract:
In Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), ion motion trapped in the FT-ICR cell is essentially a superposition of three modes : cyclotron motion, magnetron motion and *z* motion. However, in practice, 10^3 to 10^6 different *m/z* ions are simultaneously trapped and excited in the cell and the ion cloud distribution consisting of each *m/z* ion ensemble and its temporal variation are quite complicated. Moreover at high ion density, the Coulomb interactions between ions considerably influence the ion cloud distribution. In a previous work [1], a special-purpose computer board (MD-One/E, Gazogiken) for many-body problems was combined with a single processor PC. The computer board makes it possible to calculate the summation routine of the ion-ion Coulomb interaction force components in high speed. Thereby in the case of 1,000 simulation particles the computation speed became 10 times as fast as a single processor only. The extended computing configuration was applied to the simulation for the influence of ion-ion Coulomb interactions on FT-ICR mass spectra measured especially at a high magnetic field. Further, the influence of ion-ion Coulomb interactions on Xe isotopic ion mass spectra was preliminarily simulated [2].
In the present work, a special-purpose computer GRAPE-6 (for "GRAvity PipE") that is a more powerful accelerator board for many-body problems was employed and the computing configuration was further accelerated. In the case of 10,000 simulation particles the calculation speed has been 11 times as fast as MD-One/E. On the accelerated configuration Coulomb-interaction-induced effects on Xe isotopic ion mass spectra were in more detail investigated using up to 10,000 simulation particles. The simulation particle number was divided and assigned to each of seven Xe isotopic ions : $^{128}Xe^+$, $^{129}Xe^+$, $^{130}Xe^+$, $^{131}Xe^+$, $^{132}Xe^+$, $^{134}Xe^+$, and $^{136}Xe^+$ in proportion to the respective isotopic ratios. Ion excitation was performed by frequency sweep at a magnetic field of 5 T and the Xe isotopic ion spectra were simulated as a function of total ion population initially trapped in the FT-ICR cubic cell. From the simulation results, in the range up to an initial ion population of 10,000 ions, the ratios of Xe isotopic ion peak intensities appear to be consistent with respective Xe isotopic ion abundances initially trapped. However, at high ion density the ratios are not maintained. Further from analysis of temporal variations for the ion cloud distributions during detection period it has been found that Coulomb-interaction-induced dephasing of the coherent ion cyclotron motion excited significantly progresses with initial ion population in the range of more than 10,000 ions.

References

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2) M. Fujiwara, *et al*., Abstr. of the 58th Annual Conference on Mass Spectrometry, Tsukuba, Japan & 1st Asian and Oceanic Mass Spectrometry Conference, 17P-024, 188 (2010).

PWe-193 Analysis of flavonoids by Graphene-Based SALDI-TOF MS

11:10 – 12:20

Min-Wei Chien[1], Ching-Yuan Su[2], Lain-Jong Li[3], Chien-Chen Lai[1]

[1]Institute of Molecular Biology,National Chung Hsing University, Taiwan, [2]Department of Electronic Engineering, Chang Gung University, Taiwan, [3]Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan

Keywords:
flavonoids, SALDI, graphene,

Novel aspects:
Flavonoids were spotted onto a matrix of graphene-based nanoparticles and analyzed by LDI-TOF-MS

Abstract:
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a simple and fast technique for the analysis of large biomolecules but is not suitable for the detection of low molecular weight molecules and compounds, such as flavonoids and phenylpropanoids, mainly due to the lack of an appropriate matrix. Flavonoids and phenylpropanoids, such as coumarin and its derivatives, have attracted much attention recently because of their pharmacological activities and putative therapeutic benefits. In this study, we developed a quick and simple LDI-TOF-MS method for detection of flavonoids and derivatives of coumarin. Analytes were spotted onto a matrix of graphene-based nanoparticles and then analyzed by LDI-TOF-MS in the negative ion mode. Analysis of the sensitivity and effect of different graphene-based nanoparticles including graphene, graphene oxide, and reduced graphene oxide on desorption/ionizat ion of analytes showed that graphene oxide was the most suitable matrix. Moreover, we found that graphene oxide sheets of larger lateral size resulted in better desorption/ionization efficiency. Overall, we show that graphene oxide is a useful matrix for analysis of flavonoids and the derivatives of coumarin by LDI-TOF-MS in the negative ion mode.

PWe-194

13:30 – 14:40

Quantification of eight catechins and caffeine in black, white and green tea samples using UHPLC coupled to tandem mass spectrometry

Karolina Skraskova, Michaela Mudrova, Hana Vlckova, Lucie Novakova

Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Hradec Kralove, Czech Republic

Keywords:
LC-ESI-MS/MS, UHPLC, tea, catechins, caffeine

Novel aspects:
New UHPLC-MS/MS method using mixed-mode sorbent for determination of eight catechins and caffeine in white, green and black tea samples.

Abstract:
Tea infusions are rich in polyphenolic compounds *catechins*, which are responsible for the antioxidant activity and the overall positive effect of tea on human health. Furthermore the tea's energizing effect caused by the presence of caffeine is well known. Actual content of catechins and caffeine is recognized as one of the tea quality indicator. According to the treatment of the tea leaves during processing, three fundamental types are distinguished : green tea (non-fermented) , black tea (fully fermented) and white tea (partially fermented) . The fermentation process significantly decreases the content of catechins.

In this project a method for quantitative determination of eight catechins : (+) -catechin (C) , (-) -epicatechin (EC) , (-) -catechin gallate (CG) , (-) -epicatechin gallate (ECG) , (-) -gallocatechin (GC) , (-) -epigallocatechin (EGC) , (-) -gallocatechin gallate (GCG) , (-) -epigallocatechin gallate (EGCG) and caffeine (Caff) in tea samples has been developed. The analytes were separated on Acquity CSH C18 (2.1 x 100 mm, 1.7 µm) column. The separation was completed within 3.5 minutes. The mass spectrometer was equipped with electrospray ionization source and triple quadruple analyzer. The polarity switching mode was employed. The quantification was based on selected reaction monitoring.

To prepare the tea infusions, approximately 1 g of accurately weighed dry tea sample was extracted with 100 mL of hot (80 °C) water for 30 minutes. The cooled down infusion was filtrated through 0.22 µm PTFE filter. Afterwards the samples were diluted 10- and 1000-times and used for determination of the analytes.

System suitability test (SST) , method linearity range, limits of quantification and detection, accuracy and precision were assessed. The method was linear within the range 0.1 ng/mL and 500 ng/mL, with the linear coefficients being typically 0.995. The limit of quantification was set as the lowest point of the above given range. Accuracy was evaluated by spiking diluted tea extracts with standard solution mixture. The precision was determined as intra- and inter-day repeatability. All the validation parameters were evaluated individually for black, white and green teas. The matrix effects were assessed by comparison of the slopes of matrix matched calibration curves and standard calibration curve. The method was used to monitor the content of catechins and caffeine in various tea samples.

The authors acknowledge the financial support of the project SVV 265 002.

PWe-195
11:10 – 12:20

Low-energy ion scattering (LEIS) study of Cs-sputtering effects on III-V semiconductor hetero-structures during SIMS analysis

Helena Tellez[1], David M McPhail[1], Jose M Vadillo[2]

[1]Imperial College, London, UK, [2]University of Malaga, Malaga, Spain

Keywords:
Low-energy ion scattering, ToF-SIMS, semiconductor, Cs implantation, ion-solid interaction

Novel aspects:
This work presents a novel approach to study Cs surface retention and ion-sputtering effects in ToF-SIMS analysis of semiconductor hetero-structures by low energy ion scattering (LEIS) .

Abstract:
Secondary ion mass spectrometry (SIMS) has been widely applied in materials science for surface and interface characterization. Furthermore, materials properties are strongly affected by their surface and interface morphology and chemistry. In this sense, SIMS has been successfully applied in the semiconductor industry for the analysis of the surface and interface chemistry, providing outstanding depth resolution and sensitivity. In this work, we show the analysis of III-V semiconductor hetero-structures by ToF-SIMS to study the diffusion and oxidation phenomena that might take place during the multi-layered growth.
Nevertheless, additional ion-sputtering effects during SIMS analysis alter the surface and interface chemistry by atomic mixing and ion implantation. These effects are especially relevant when using chemically-active primary species, such as Cs^+, which leads to modifications of ionization probabilities and sputter yields due to the implantation of primary ions on the surface and near-surface regions and the subsequent material work function reduction (1) . Since Cs is very volatile and reactive, Cs retention is also dependent on oxygen availability in the ultra-high vacuum chamber and/or material surface (2) . Therefore, in order to understand the Cs retention on the surface, low-energy ion scattering (LEIS) was used to probe the outermost surface during ToF-SIMS analysis. In addition to the first mono-atomic layer specificity, quantitative LEIS analysis of Cs coverage can be performed when correcting for work function modifications (3) . In this case, the possibility of performing the LEIS analysis in the same instrument without exposing the surface to the atmosphere, allows us to avoid any further oxidation/contamination surface to accurately estimate the surface coverage as Cs dose is building up during ToF-SIMS analysis.

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PWe-196
13:30 – 14:40

Quality control of multilayered/nanoheterostructured solar cells by secondary ion mass spectrometry and ion beam etching techniques

Jose M Vadillo[1], Diana J Padilla[1], Helena Tellez[2], John W Druce[2], David S McPhail[2], Jose J Laserna[1]

[1]University of Málaga, [2]Imperial College

Keywords:
SIMS, FIB, solar cells analysis

Novel aspects:
The use of SIMS (quadrupole and ToF) and ToF-SIMS imaging over FIB sections to perform depth-resolved analysis with nanometric resolution of multilayered/nanostructured solar cells.

Abstract:
High efficiency solar cells represent the preferred photovoltaic component on spacecrafts and will be used at long term for many industrial applications. Its technology requires a continuous development of new materials and fabrication processes. In the particular case of III-V solar cells, the alloy composition determines its energy band gap and many III-V alloys can be grown epitaxially on each other, generating a stack of multiple layers (some of the as small as 5 nm) of different band-gap that must manufactured under strict control of composition, properties and thickness. Secondary ion mass spectroscopy (SIMS) represents an excellent technique for a complete characterization of a multilayered solar cell device due to its depth-profiling capabilities and sensitivity. The communication will show examples of quality control of III-V solar cells using quadrupole SIMS, and ToF-SIMS where defects associated to the manufacturing process have been identified. As a complementary technique, Focussed Ion Beam (FIB) sections of the structure were obtained and subsequently mass-selectively imaged by ToF-SIMS.

PWe-197

11:10 – 12:20

Ultra high pressure comprehensive two-dimensional liquid chromatography combined with hybrid mass spectrometry for the elucidation of carotenoids in chilired peppers

Marcus Mreyen[1], Francesco Cacciola[2,3], Paola Donato[3,4], Daniele Giuffrida[5], Germana Torre[3], Paola Dugo[3,4], Luigi Mondello[3,4]

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Keywords:
comprehensive LC, Ion trap, TOF, accurate mass,

Novel aspects:
Combination of high sophisticated LCxLC techniques with advanced hybrid type mass spectrometry for the structural elucidation of natural compounds

Abstract:

Introduction

Some of the important ingredients in paprika extracts are carotenoids formed in the fruit during ripening. These compounds are not only used in the food industry but also in pharmaceutical and cosmetic products. A total of 33 different compounds have been separated and identified in red chilli peppers.

A comprehensive normal-phase × reversed-phase (NP-LC×RP-LC) liquid chromatographic system was developed, and applied for analysis of the intact carotenoid composition of chili red peppers, with PDA (photodiode array) and MS (mass spectrometry) . The combination of ion trap technology coupled with accurate mass measurements was used for the identification and classification of the different compounds.

Methods

A micro-bore cyano column (250x1.0 mm, 5 mm d.p.) was chosen for the first dimension separation, interfaced to a secondary (2D) C18 column (30x4.6 mm, 2.7 mm d.p.) packed with fused-core particles. Subsequently, two columns of the same stationary phase were coupled serially for second dimension separation, and operated under UHPLC (ultra high pressure LC) conditions, within a cycle time of 90 or 60 sec. The advantages of the latter set-up over the single-column one are demonstrated by the higher peak capacity values (nc 836 or 1325 vs. 571) ; especially for the separation of the more polar chemical classes (mono-esters and free carotenoids) , better focusing at the head of the 2D column resulted in improved separation.

Poster Session

Wedensday, 19th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PWe-198 Identification of Organic Contaminants on Silicon Surface by Mass Spectrometry

13:30 – 14:40

Yoon Mi Lee, Sang Yoon Shin, Jung Dae Park, Pil Kwon Jun, Jong Soo Kim

Semiconductor Business, Samsung Electronics / Gyeonggi-Do, Korea

Keywords:
Semiconductor, Organic Contaminants, TD-GC/MS, TOF-SIMS, LDI-TOF

Novel aspects:
This work shows the differences in detection sensitivity of various mass spectrometry and new application using LDI-ToF MS on semiconductor.

Abstract:
Increasing yield loss due to non-visual defects and process variations requires new approaches in methodologies, diagnostics and control. Especially, Organic contaminants such as organophosphates, antioxidants, plasticizers, caprolactam, phthalates or siloxanes on wafers can cause unwanted doping, poor gate oxide reliability, delamination, contact resistance, solder/copper electroplating failure, mis-align, optical defects, wetting problems, silicon nitride nucleation delays, ellipsometry errors and many other thin film issues that can reduce yield. For optimum process control, organic contaminants have to be under control.
This research investigated organic contaminants on silicon wafers for semiconductor. During the semiconductor process, trends of defect were increased at certain steps. We matched the system of defect trends and then discovered thinner chemical that is adhesion promoter for photo process caused defects. For simulation, thinner was coated on the clean wafer. The defect like the whirlwind was formed on clean wafers after treating the thinner. C, O, S, Fe and Al elements were characterized by Energy Dispersive X-ray analysis (EDX) . However, we couldn't observe any differences between normal and abnormal thinner chemical about metal impurities. Thermal Desorption GC-MS (TD-GC/MS) analysis was performed that is typical analysis for identifying the organic contaminants and matching to materials outgassing results to determine the root source of the organic contaminants. However, any special chromatogram was not detected. Thinner contaminated wafers were examined by using Laser Desorption/Ionization Time of Flight Mass Spectrometry (LDI-ToF MS) and Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) . ToF-SIMS result shows the NH_4^+, CH_4N^+, $C_2H_6N^+$, $C_4H_{12}N^+$ ions and LDI-ToF MS result shows 73.9Da in abnormal chemical. According to these results, tetramethylammonium hydroxide (TMAH) is contaminated in thinner chemical. To convince these results, 2.4 ppm TMAH coated wafers were investigated and 73.9Da was detected by using LDI-ToF Method. These wafers were also investigated by using TD-GC/MS (Gerstel TDS 2 thermodesorption system) . However, sufficient signal was detected after concentrating 100 times. TMAH is usually used as an anisotropic etchant of silicon. It is also used as a basic solvent in the development of acidic photoresist in the photolithography process. In this case, when mixing the chemicals TMAH was not a component of thinner. We discovered the cleaness of the environment was not controled about organic materials for semiconductor grade.

Poster Session

Wedensday, 19th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PWe-199

11:10 – 12:20

Comprehensive Targeted Compound Identification Using Pseudo-Precursor Ion Scan and Neutral Loss Scan for Degradation Analysis of Electrolytes

Kaoru Karasawa, Toshiyuki Yamazaki, Sumie Ando

ABSCIEX

Keywords:

QqTOF, comparative study, structure elucidation, pseudo-precursor ion scan and neutral loss scan, impurity and degradation analysis

Novel aspects:

Various pseudo-precursor ion scan and neutral loss scan data are available easily and comprehensively from acquired high speed and comprehensive IDA data.

Abstract:

Introduction:

Li-ion batteries and capacitors are being developed for use in mobile phones, electric vehicles, as well as energy storage especially from natural resources. For development of Li-ion batteries and capacitors, it is important to evaluate their components of starting materials, products, and ageing effects. In addition, analysis of electrolyte started gaining attention ; however the method is not fully established because of sample stability, complexity, strong acidity and others. To establish the analytical methods, we accomplished comparative studies of electrolytes in Li-ion batteries and structural elucidation of characteristic electrolyte additives and products in cycle test (reported JSMS and Battery Symposium in Japan last year) . In this report, we perform further degradation analysis and comprehensive targeted compound identification using pseudo-precursor ion scan and neutral loss scan technique on acquired high speed and comprehensive IDA data.

Material and Methods:

Samples : 35 electrolytes and blank acetonitrile was used as samples. Each electrolyte was extracted with acetonitrile from 35 different cells from six Li ion batteries (#1 to #5) after discharge and charge cycle tests (0, 100, 500 cycles) and diluted ten times with acetonitrile before injection.

HPLC Condition: System : Shimadzu UFLC LC System (Shimadzu Corp, Japan) , Column : Acquity HSS T3 2.1X100mm, 1.8mm (Waters) , Mobile Phase : gradient elution with A : 10mM ammonium acetate in water and B : 20 % THF in acetonitrile, Flow Rate : 0.500mL/min, Injection Volume : 2 µL, Column Temperature : 40° C, Total Runtime : 19.5min

MS Condition: System : AB SCIEX TripleTOF™ 5600 LC/MS/MS System (AB SCIEX, Canada) , Ionization : ESI, Polarity : Positive and Negative, Method : IDA (Information dependent acquisition, Survey Scan 100msec and 5 or 10 or 25 Product Ion Scan 50, 25, 10msec each, DBS (dynamic background subtraction) ON) , Cycle Time : 0.4sec, CE : +/-35+/-15V

Software: Acquisition : Analyst® TF1.5.1 Software, Analysis : PeakView® Software (AB SCIEX, Canada) for structure elucidation and pseudo-precursor ion scan and neutral loss scan, MarkerView™ 1.2.1.1 Software (AB SCIEX, Canada) for multivariable analysis (PCA-DA, t-Test) , MetabolitePilot™ 1.5 Software (AB SCIEX, Canada) for analogue identification and sample comparison

Results and Discussion:

* The compounds related to degradation and life time of batteries were identified using PCA-DA of MS data with the results of discharge and charge cycle tests and MS/MS data.

* A large number of analogues of these compounds were identified individually, using pseudo-precursor ion scan and neutral loss scan from one high speed and comprehensive IDA data.

* The intensities of these compounds were corresponded to the results of cycle tests. It suggests that these analogues affect the performance of batteries.

With high speed IDA with DBS, MS/MS is acquired for almost all components in one run, and additional MS/MS run is not required. Various pseudo-precursor ion scan and neutral loss scan data are available easily and comprehensively from the acquired data.

This technique is applicable to and powerful tool for impurity and degradation analysis, and other applications.

Poster Session

Wedensday, 19th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PWe-200

13:30 – 14:40

Analysis of degradation products in electrolyte for rechargeable lithium-ion battery through high mass accuracy MSn and multivariate statistical technique

Hiroki Nakajima, Yamaki Satoshi, Nishine Tsutomu, Furuta Masaru

Shimadzu Co., Kyoto, Japan

Keywords:

LCMS, high mass accuracy MSn, lithium-ion batteries, electrolyte, multivariate statistical technique

Novel aspects:

Formulae and structure of degradation products in the electrolyte were identified using high mass accuracy MSn and multivariate statistical technique.

Abstract:

Rechargeable lithium-ion batteries are one of the major power sources for portable electronic devices and electric vehicles because of their high voltage and high energy density. The electrolyte of a lithium-ion battery is consisting of a lithium salt in an aprotic organic solvent. The typical operational potential of a lithium-ion battery is between 0 and 5 V. Therefore, solvent can be reduced or oxidizedat the negative and positive electrodes during the battery charging process. As a result, various degradation products are generated in electrolyte, and cause some problems such as a decrease in the capacitance of battery. Here, we present the analysis method of degradation products generated in electrolyte using high mass accuracy $MS^n$and multivariate statistical technique.

The electrolyte was a mixture of ethylene carbonate (EC) and dimethyl carbonate (DMC) (EC : DMC = 1 : 2 vol%) containing 1 M lithium hexafluorophosphate ($LiPF_6$) .The electrolyte A taken from unused lithium-ion battery and the electrolyte B taken from lithium-ion battery repeated charge and discharge cycles (200 times) were used as samples. Those samples were prepared 1/10 dilution with methanol for LCMS-IT-TOF (Shimadzu Corporation) measurement. Principal component analysis (PCA) was performed using data acquired by LCMS-IT-TOF measurement of electrolyte A and electrolyte B (n=3) to find the compounds generated in electrolyte B. Then, those compounds were identified chemical formula using software "FormulaPredictor " (Shimadzu Corporation) . SIMCA-P+ (Umetrics) and Profiling Solution (Shimadzu Corporation) were used for PCA.

On the score plot of PCA, the group of electrolyte A and electrolyte B were located at left side and right side, respectively. 13 unique ions of electrolyte B were observed at right side on the loading plot. And, those ions were not detected on the extracted ion chromatogram (EIC) of electrolyte A. These results suggested that those ions were degradation products generated in the electrolyte of lithium-ion battery repeated charge and discharge 200 cycles. The formula of the ion (*m/z* 284.0982) being one of 13 unique ions of electrolyte B was predicted as $C_9H_{14}O_9$ (ionic species ; $[C_9H_{14}O_9+NH_4]^+$) using high mass accuracy MS^1 data and formula predictor. Indeed, the structure of $C_9H_{14}O_9$was predicted as $H_3C-(OCO_2-C_2H_4)_2-OCOCH_3$ referring to some articles on degradation products in electrolyte. We also measured MS^n of the ion (*m/z*284.0982) to determine the validity of the predicted chemical structure. The result of MS^3 data and neutral loss ions showed that the predicted structure of $C_9H_{14}O_9$was correct. By the same method, formulae and structures of other 12 ions also were identified as $C_4H_{11}O_5P$,$C_8H_{18}O_5$, $C_3H_9O_4P$,$C_{10}H_{22}O_6$, $C_7H_{15}O_8P$,$C_9H_{12}O_9$, $C_{12}H_{26}O_7$,$C_6H_{10}O_6$, $C_6H_{13}O_7P$,$C_{16}H_{34}O_9$, $C_{16}H_{34}O_9$,and $C_{12}H_{18}O_{12}$. It was cleared that the chemical species of degradation products generated in the electrolyte with increasing charge and discharge cycles were carbonate and phosphate from result of this study.

PWe-201 GC/MS Determination of N-Nitrosamines in Rubber Teats

11:10 – 12:20

Peng Gao, Jun Fan, Li X Deng, Hong T Huang

SHIMADZU(CHINA)CO.,LTD,ShangHai,China

Keywords:

N-Nitrosamines；Rubber Teats；Solid Phase extraction

Novel aspects:

A new GC/MS method for the determination of N-Nitrosamines in rubber teats, comparing with traditional GC/TEA method.

Abstract:

A method of GC/MS was developed for the determination of 12 N-nitrosamines (N-nitroso dimethylamine, N-nitroso methylethylamine, N-nitroso diethylamine, N-nitroso dipropylamine, N-nitroso dibutylamine, N-nitroso-N-methylaniline, N-nitroso N-ethylaniline, N-nitroso piperidine, N-nitroso pyrrolidine, N-nitroso morpholine, N-nitroso diphenylamine, N-nitroso dibenzylamine) in rubber teats. The release of N-nitrosamines from rubber teats were accomplished by ultrasonic extraction and then purified by C18 SPE cartridges. The analyses were separated on Stabilwax capillary column and detected by EI ion source with selected ion mode. Good linearity of calibration curve was obtained in the concentration range from 50 to 1000 μg/L for all compounds examined. Recoveries ranged from 70 % to 110 %, with RSD (n=5) less than 10 %. The limits of detection (3 S/N) of 12 N-nitrosamines examined ranged from 0.15 to 0.61 μg/Kg.

PWe-202 Analysis of black tea by GC-MS and high-resolution UPLC-MS

13:30 – 14:40

Christian Grün, Boudewijn Hollebrands, Julien Boelhouwer

Unilever R&D, Vlaardingen, The Netherlands

Keywords:

Black tea, Camellia sinensis, UPLC-TOF-MS, GC-MS, phytochemicals

Novel aspects:

The method described allows the differentiation between different black teas, which is particularly challenging due to their complexity. By combining GC-MS with UPLC-TOF-MS, several compound classes can be analyzed simultaneously.

Abstract:

After water, tea (*Camellia sinensis*) is the most consumed beverage in the world. Of all tea produced, most is consumed as fully fermented (black) tea. Still, the diversity in between different black teas is enormous, which is on the one hand based on geographic regions of tea plant cultivation and on the other hand on the procedures used for withering and fermenting of tea leaves.

Here, we use gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-TOF-MS) to determine the phytochemical diversity between three common black teas. The teas were selected on their geographic origins (Yunnan - China, Assam - India or a tea blend) and on differences in the fermentation processes (orthodox versus CTC) . GC-MS was used in electron ionization mode to identify small phytochemicals such as organic acids, amino acids, and monosaccharides. UPLC-TOF-MS was used in positive and negative electrospray mode and enabled the detection of polyphenols such as catechins, chlorogenic acids and flavonol glycosides and other classes of phytochemicals such as carbohydrates and peptides.

By combining GC-MS with UPLC-TOF-MS, a large range of components was covered, enabling the detailed differentiation between the three black teas. Both GC and UPLC exhibit impressive chromatographic resolving power required for the separation of complex mixtures such as black tea. Identification was accomplished by comparison with libraries and by making use of accurate mass data.

The data indicate that despite the differences in origin and processing between the black teas, remarkable similarities in phytochemical composition exists. Detailed analysis shows however, that the levels of individual phytochemicals present in black tea can vary tremendously. Together, the data indicate that the combination of GC-MS and UPLC-TOF-MS forms a strong tool in differentiating between different black teas.

Plenary Lecture

Thursday, 20th September

Main Hall

08:00 – 08:45

Plenary Lecture 4: Hisayoshi Yurimoto

Chair: Robert J Cotter (Johns Hopkins University School of Medicine, USA)

PL4-0800 Science of asteroid sample return mission “HAYABUSA“

08:00 – 08:45

Hisayoshi Yurimoto
Hokkaido University, Sapporo, Japan

Keywords:
SIMS, isotope, Asteroid

Novel aspects:
We measured the first asteroidal sample obtained by a planetary exploration HAYABUSA, demonstrating that the most abundant meteorite species fallen into the Earth come from S-type asteroids.

Abstract:
Of the ˜40,000 meteorites we know of, only 14 have had their pre-impact orbits ascertained. The aphelia of these 14 orbits are located within the Main Asteroid Belt between Martian and Jovian orbits, which suggests that meteorites are an asteroidal origin. However, telescopic spectroscopy of asteroidal surfaces had raised serious doubts on the hypothesis. One of the primary scientific purposes of Hayabusa mission was demonstration of this hypothesis.
The Hayabusa spacecraft made two touchdowns on the surface of asteroid 25143 Itokawa on November 20th and 26th, 2005. After recoveries from serious accidents on the way home from Itokawa, the spacecraft made the reentry into the terrestrial atmosphere on June 12th, 2010 and the sample capsule was successfully recovered in Australia on June 13th, 2010.
More than 1,500 grains were found in the sample capsule and identified as rocky particles. Although their sizes are mostly less than 10 micrometers, some larger grains of about 100 micrometers or larger were also included. About 50 particles among the larger grains were subjected to one set of preliminary examinations. The preliminary examinations started from January 21st, 2011. The examinations include X-ray CT analysis, X-ray diffraction analysis, petrology, mineral chemistry, oxygen isotope analysis, trace element analysis and noble gas analysis.
In order to determine extraterrestrial origin of the particles in the capsule, isotope analysis of oxygen for individual particles is essential because extraterrestrial materials usually show different isotope ratios from terrestrial materials. However, the degree of difference is less than 1 %. A precise analysis technique by SIMS was newly developed and applied.
The SIMS characterization of Itokawa particles certified that Itokawa was composed of similar materials of ordinary chondrites. This is the first direct link showing that S-type asteroids is one of the sources of the ordinary chondrites, which are the most abundant meteorite species fallen into the Earth. From this citification, we confirm that insights based on meteorite research are the right way to study origin of our solar system. New insights for the early solar system are expected by further detail measurements of Itokawa particles.

Oral Session

Thursday, 20th September

Main Hall

09:00 – 11:00

Session 31: Native Mass Spectrometry and Structural Biology

Chair: Satoko Akashi (Yokohama City University, Japan)

S31-0900

09:00 – 09:40

[Keynote Lecture] Integrating Native Mass Spectrometry and Top-Down MS for Defining Protein Interactions Important in Biology and Medicine

Joseph A Loo

University of California-Los Angeles, USA

Keywords:
native MS, top-down MS, ESI

Novel aspects:
Protein binding dynamics and topology can be provided by native mass spectrometry.

Abstract:
The application of mass spectrometry (MS) and ion mobility for studying proteins and protein complexes has utility in structural biology and biomedical research. The assessment of protein interactions can address the functional role of proteins and protein complexes. Measuring the molecular masses of protein complexes reveals information on stoichiometry of binding and identification of interacting partners. MS and ion mobility measurements can supply information on the structural aspects of gas-phase/solution-phase protein conformations and supramolecular assemblies in excess of 1 MDa. However, MS can advance an additional layer of information that is critical to structural biology : location (or topology) and dynamics.

Developments in protein MS and tandem MS (MS/MS, or top-down MS) to define the structures of native protein complexes, including the sites of ligand bonding, will be discussed. Collisionally activated dissociation (CAD) of gas-phase stable noncovalent complexes resulting from electrostatic interactions can be used to probe ligand-binding sites (e.g., nucleotides, metals) . Electron transfer dissociation (ETD) and electron capture dissociation (ECD) can probe the binding sites of weakly bound ligands and the topology of protein-protein complexes (e.g., hemoglobin) . We are using ECD-Fourier transform ion cyclotron resonance (FTICR) MS to investigate the molecular action of compounds that prevent amyloid fibril formation in neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

Native proteins and complexes are not necessarily static entities, as they are in constant motion and perhaps changing their partners during the "dance " that defines their roles in biological processes. We are using native MS and top-down MS to decipher the dynamics of protein interactions that govern iron-capture by pathogens. Bacteria need iron from the host to establish infection. The blood infection from *Staphylococcus aureus* affects a large fraction of the human population and its pathological mechanism has not been fully elucidated. It has been postulated that a receptor on the surface of *S. aureus*, IsdH, is involved in the capture of heme from hemoglobin (Hb) and this is the source of iron required for infection. Native ESI-MS and MS/MS are used to study the dynamics of noncovalent interactions between Hb and the IsdH protein receptor. The complex processes of site-specific protein recognition and heme transfer, including the kinetics of multiple binding equilibria, can be monitored by ESI-MS to provide important clues to the mechanism of *S. aureus* infection. This information could be useful for designing new compounds and strategies for fighting and preventing potentially lethal *S. aureus* infections. Moreover, it demonstrates the ability of native ESI-MS for providing a view of the dynamics of protein-ligand assembly-disassembly that would be difficult to measure by other biophysical methods.

*Acknowledgments:*Support from the US National Institutes of Health (R01 RR20004, S10 RR023045, S10 RR028893) is acknowledged.

Oral Session

Thursday, 20th September

Main Hall

09:00 – 11:00

Session 31: Native Mass Spectrometry and Structural Biology

Chair: Satoko Akashi (Yokohama City University, Japan)

S31-0940

09:40 – 10:00

Ion mobility-mass spectrometry for the study of conformational space and topology of proteins and protein-DNA complexes

Frank Sobott

Antwerp University, Antwerp/Belgium

Keywords:
ion mobility, native mass spectrometry, protein conformation, protein complexes, protein-DNA complexes

Novel aspects:
Use of native MS and ion mobility to determine protein conformational space and topology of protein-protein and protein-DNA complexes

Abstract:
While established techniques in structural biology such as x-ray crystallography can reveal biomolecular structure in unsurpassed detail, they only highlight certain aspects of the different conformational and association states of a protein. Other methods such as NMR are either not well suited to large, dynamic structures, or they lack the detail necessary (e.g. EM) to resolve individual species in heterogeneous conformational or association equilibriae.
Native MS as a'single molecule technique'can cope well with such heterogeneity, and in combination with ion mobility spectrometry, different conformational states can now also be resolved reasonably well. But what does a measured collision cross section really tell us about the conformational space of a protein or the subunit arrangement of a complex? How important is the absence of bulk solvent, and how much do the measured size and the flexibility depend on the internal energy (temperature) of the ions?

We are using native nano-ESI MS coupled with T-wave ion mobility spectrometry (Waters Synapt G1 and G2) to assess the structure and conformational space of a couple of well understood proteins and complexes, and correlate this data with results from other biophysical methods such as size-exclusion chromatography, surface plasmon resonance and small-angle x-ray and neutron scattering (SAXS and SANS). In side-by-side comparison of closely related protein sequences (e.g. with and without mutations, truncations or post-translational modifications), such subtle changes are frequently reflected in the position and width of the drift time profile (i.e. 'size' and 'flexibility' of the ion). We will correlate these data with candidate structures of the proteins and complexes.

The binding of ligands or counter-ions however can cause a considerable compaction of the gas-phase structure, as will be demonstrated with an example. For multiprotein complexes it is an often reported phenomenon that a systematic variation of instrumental parameters which affect the internal energy of the ions (such as voltage offsets and background gas pressures) leads to an intial gas-phase collapse of hollow structures such as in the hexameric ring discussed here before further increase of the internal energy leads to extended, but still oligomeric conformations of the complex. In this way, we can for example determine the threshold of unfolding or the 50% unfolding point as an indirect measure of the gas-phase stability of native biomolecular structures.

Taken together these data highlight the importance of controlling the internal energy of ions carefully in the experiment, and raise some critical points regarding the measurement of absolute (rather than relative) sizes by IM-MS. They also demonstrate the level of information we can extract from careful, systematic measurements of collision cross sections of ions of biochemical and pharmaceutical interest.

Oral Session

Thursday, 20th September

Main Hall

09:00 – 11:00

Session 31: Native Mass Spectrometry and Structural Biology

Chair: Satoko Akashi (Yokohama City University, Japan)

S31-1000

10:00 – 10:20

Structure of a Beta-Crystallin Heterodimer by Ion Mobility and Radical Probe Mass Spectrometry

Kevin Downard[1], Yuichi Kokabu[2], Mitsunori Ikeguchi[2], Satoko Akashi[2]

[1]University of Sydney, [2]Yokohama City Unversity

Keywords:
crystallin, protein complex, ion mobility, radical probe, homology modeling

Novel aspects:
First application of protein footprinting by RP-MS in concert with ion mobility mass spectrometry and homology modeling to characterize a protein complex

Abstract:
Beta-crystallins constitute the major proteins of the lens of the eye in mammals. Their association with one another and other crystallins is critical for lens clarity and vision. The dimerization of beta-crystallins is first step in the formation of many higher order complexes, yet remains poorly understood at the molecular level. We report on the application of Ion Mobility and Radical Probe Mass Spectrometry, in conjunction with homology modeling, to characterize the structure of the βB2B3-heterodimer.
NanoESI-TOF and IM-MS were performed on a Waters SYNAPT-G2 mass spectrometer to identify protein dimers in a solution of beta-crystallin. The collision cross sections (CCS) of the B2 monomer, and B2B2 and B2B3 dimers were measured and found to be similar in value at 3121Å 2 and 3165Å 2 respectively. The exclusive presence of beta-B3-crystallin within the B2B3-heterodimer prompted its analysis by RP-MS. The level of oxidative modification at amino acid residue side chains across the B2 and B3 crystallin proteins was established by LC-MS. This data established a solvent accessibility profile of B3 in complex with B2. Oxidation levels ranging from 0-100% were observed across the reactive residues of the βB3 subunit. Several homology-modelled structures for the heterodimer were then constructed and assessed in terms of their calculated CCS and whether the solvent accessibilities were in accord with measured oxidation levels. Two homodimeric structures derived from the βB2-tetramer structure (PDB 2BB2) are possible containing two of the βB2 subunits along the PQ plane in closest proximity (AB) and along the RQ plane (AD). Due to high sequence identity among the B2 and B3 subunits, these structures were used to model both a AB and AD βB2B3-heterodimer. The calculated CCS's for the lowest energy structures of the βB2B3 AB and AD heterodimers had average values of 2888 and 3140 Å 2 respectively. Two additional homology models were constructed. One "half" model used two N and two C-terminal domains extracted from four separate chains (A-D) positioned side-by-side in one half of the βB2-tetramer. The connecting loops were generated from the structure of the βB3- homodimer structure extracted from the human βB3-hexamer (PDB 3QK3). In the final "twisted " model, a βB2 monomer (A chain) extracted from the tetramer was coupled to the N-terminal domain extracted from chain B and C-terminal domain from chain D. These two βB2 domains were connected by elongation of the linker region and the resultant subunit replaced with the βB3 subunit. The homology modelled structure for the AD βB2B3-heterodimer was found to be most consistent with the IM-MS and RP-MS protein footprinting data.

Oral Session

Thursday, 20th September

Main Hall

09:00 – 11:00

Session 31: Native Mass Spectrometry and Structural Biology

Chair: Satoko Akashi (Yokohama City University, Japan)

S31-1020

10:20 – 10:40

Application of Backbone Amide Hydrogen/Deuterium Exchange-Mass Spectrometry (HDX-MS) to Characterize Protein-Ligand Interactions and Protein-Protein Interactions

Yoshitomo Hamuro

ExSAR, Monmouth Junction, NJ, USA

Keywords:
hydrogen/deuterium exchange, protein-ligand interactions, epitope

Novel aspects:
Backbone amide hydrogen/deuterium exchange-mass spectrometry (HDX-MS) was used to investigate kinase-ligand interactions and to map conformational epitopes of antigen-antibody interactions

Abstract:
This presentation will discuss the application of backbone amide hydrogen/deuterium exchange coupled with proteolysis, liquid chromatography, and mass spectrometry (HDX-MS) to investigate protein-ligand interactions and protein-protein interactions at the sub-molecular level. HDX-MS is an increasingly popular, widely-applicable, medium-resolution, and medium-throughput technology for protein dynamic characterization. The presentation consists of three parts : introduction to HDX-MS, the application of HDX-MS for protein-ligand interactions, and the application of HDX-MS for protein-protein interactions.

The first part explains the physicochemical background of HDX-MS methodology and an overview of how the method works. In the HDX-MS work flow, an analyte protein is first mixed with deuterated buffer and the reaction mixture is incubated for a predetermined duration. Next, the addition of acidic buffer quenches the exchange reaction and then exchanged and quenched protein is flowed over a protease column. The peptic fragments will be desalted and separated by a reverse phase column. Finally, mass analysis detects the change in mass upon the exchange reaction. When a protein-protein interaction is studied by HDX-MS, at least two sets of experiments are carried out : the HDX-MS of the unbound protein and the HDX-MS of the protein in complex with its binding partner. The HDX-MS behavior of the protein in the absence or presence of its binding partner can describe the dynamic properties of the protein in each condition at the sub-molecular level. The difference of the HDX-MS patterns can shed light on how the protein dynamics change upon complex formation.

The second part discusses HDX-MS of p38 MAP kinase, a validated cancer target, in the presence and absence of various ligands. HDX-MS results showed that all tested ligands interact with two regions of the kinase. The two regions are approximately thirty residues in total and cover both the ATP binding site and the DFG region. The presence of ATP analogs protected HDX reactions of the ATP binding site without perturbing the HDX reactions near the DFG region, while a known DFG binder retarded the HDX reactions of only the DFG region without interfere with HDX reactions at the ATP binding site. Moreover, a chemical conjugate of an ATP binder and the DFG binder protected HDX reactions at both the ATP binding site and the DFG region. The results exemplify the potential of fragment-based drug discovery by HDX-MS.

The third part illustrates how HDX-MS can be used for protein-protein interactions, particularly for epitope mapping of various antigen-antibody interactions. HDX-MS successfully mapped challenging discontinuous conformational epitopes. Understanding antigen-antibody interactions at the sub-molecular level is of particular interest for scientific, regulatory and intellectual property reasons, especially with increasing demand for monoclonal antibody therapeutic agents. While various techniques are available for the determination of an epitope, there is no widely-applicable and reliable method available, especially for discontinuous conformational epitopes. HDX-MS was used to map the epitopes of cytochrome c : E8, IL-13 : CNTO607, and IL-17A : CAT-2200 interactions. All three epitopes are discontinuous conformational epitopes and the epitopes identified by HDX-MS are in good agreement with those identified using high-resolution X-ray crystallography. Other epitope mappings for antigens with therapeutic value are also discussed.

Oral Session

Thursday, 20th September

Main Hall

09:00 – 11:00

Session 31: Native Mass Spectrometry and Structural Biology

Chair: Satoko Akashi (Yokohama City University, Japan)

S31-1040

10:40 – 11:00

An integrated workflow for structural mass spectrometry: combining X-linking technology with ionmobility for the structural characterisation of macromolecular assemblies

Florian Stengel[1], Argyris Politis[2], Zoe Hall[2], Eri Sakata[3], Helena Hernandez[2], Alexander Leitner[1], Thomas Walzthoeni[1], Carol V Robinson[2], Ruedi Aebersold[1,4]

[1]Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, [2]Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, UK, [3]Laboratory of Protein Metabolism, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo, 156-8506, Japan, [4]Faculty of Science, University of Zurich, Switzerland

Keywords:
Structural Proteomics Ionmobility Cross Linking

Novel aspects:
Novel method for studying protein complexes that combines cross linking, ionmobility and native mass spectrometry with modelling approaches in a single workflow

Abstract:
Classical methods for the determination of the structure of protein complexes are increasingly complemented by emerging experimental techniques. This is especially true if no high-resolution structure can be attained due to the transient nature of the macromolecular assembly or in cases where no pure purification of sufficiently high concentration for classical structural methods can be achieved.
Here emerging methods in structural mass spectrometry (MS) can pose an exciting alternative, due to their low sample requirements and comparatively high measuring speed.
MS of intact assemblies is able to assess the absolute stoichiometry of the intact complex and intermediate sub complexes. Chemical cross-linking coupled with mass spectrometry (CXMS) on the other hand is capable of providing multiple high resolution interactions for a single protein-protein interaction and at peptide resolution. Additionally both ion mobility mass spectrometry (IMMS) and chemical cross-linking coupled with mass spectrometry (CXMS) provide information on shape and topology. Combining the different datasets can generate complete interaction maps for subsequent merging with topological information gained from IM-MS and CXMS. Using those synergies can help to understand the stoichiometry and complete subunit interaction maps of intact complexes and when combined with computational tools, even generate 3 dimensional *ab initio* models.

Here, we incorporate for the first time MS of intact assemblies, IMMS and CXMS datasets with modelling approaches in one single workflow to generate restraints for subsequent structure determination of multi protein complexes. We trained our approach on a set of protein complexes with known high resolution structures before validating it on a protein assembly of high biological interest, the proteasome from *Saccharomyces cerevisiae*, for which still no atomic-resolution structure does exist.

Our approach presented here converts IMMS measurements into global restraints and combines them with additional local restraints imposed by CX-MS and the MS of intact complexes. Candidate models are then selected by a search for good scoring configurations that satisfy all restraints.

Such a hybrid approach can not only generate more complete interaction maps of investigated complexes and provide new insights into the structure of transient complex macromolecular machines. It should also greatly enhance the interpretation of EM densities and may become a generic pairing in its own right for high throughput structural biology.

The PPROSPECTS GrantHEALTH-F 4 -2008-201648 under the EU 7th Framework program and the Wellcome Trust (Sir Henry Wellcome Fellowship) are gratefully acknowledged for funding.

Oral Session

Thursday, 20th September

Room A

09:00 – 11:00

Session 32: Formation and Dissociation of Peptide Radical Ions

Chair: Dominic T W Chan (The Chinese University of Hong Kong, Hong Kong SAR)

S32-0900 [Keynote Lecture] Formation and Dissociation of Peptide Radical Ions

09:00 – 09:40

Roman A Zubarev

Karolinska Institutet, Stockholm, Sweden

Keywords:
hydrogen-abundant radicals, nonergodic fragmentation, peptide sequencing, fragment stability, posttranslational modifications.

Novel aspects:
An overview of the recent efforts to decipher the ECD/ETD mechanism, and an outlook for new fragmentation mechods

Abstract:
Electron capture dissociation (ECD) and electron transfer dissociation (ETD) are sister fragmentation techniques where multiply protonated molecular species capture an electron (which is free, as in ECD, or bound to an anion, as in ETD) and form unstable hydrogen-abundant radical cations. After 15 years of mechanistic studies, many questions remain unanswered. An overview will be given on the status of mechanistic investigations in ECD/ETD, with emphasis on the following questions :
1. Site of the electron attachment and its effect on the fragmentation pattern. Which process dominates : direct charge recombination or indirect process (capture, backbone cleavage, charge recombination) ?
2. What exactly is the role of fragment stability in determining the fragment abundances?
3. What is the role of secondary structure, charged and neutral hydrogen bondings in determining the fragment abundances?
4. What role does internal energy of the precursor ion play in ECD cross section?
5. What is the role of the kinetic energy of electrons in determining the fragment abundances? What determines the branching ratio between electron capture, electronic excitation and ionization?
6. What is the N-C_a fragmentation time scale upon electroncapture?
7. What is the origin of less conventional backbonecleavages, e.g. leading to radical a-ions?

S32-0940

09:40 – 10:00

Ab initio MO Study on the Fragmentation Mechanisms of Protonated Phosphopeptides in "On-Resonance" and "Off-Resonance" Pulsed Gas Introduction Collision-Induced Dissociations

Takae Takeuchi[1,2], Ayaka Takahashi[1], Erika Sugawara[1], Tomoko Kimura[1], Yuka Kurosaki[1], Shigeki Kajihara[3], Hiroko Morinaga[4], Shinichi Iwamoto[3], Koichi Tanaka[3]

[1]Department of Chemistry, Faculty of Science, Nara Women's University, Nara, Japan, [2]National Institute of Advanced Industrial Science and Technology (AIST), Ikeda, Osaka, Japan, [3]Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation, Kyoto, Japan, [4]Technical Research Laboratory, Shimadzu Corporation, Kyoto, Japan

Keywords:

"On-Resonance " Pulsed Gas Introduction CID, Fragmentation Mechanism, Ab initio Calculation, Phosphopeptide, MALDI

Novel aspects:

A new strategy of predicting fragmentation processes of protonated phosphopeptides was developed based on ab initio MO calculations.

Abstract:

Introduction

The occurrence of facile neutral losses of H_3PO_4 and/or HPO_3 from protonated phosphopeptides makes it difficult to determine both the site of phosphorylation and the amino acid sequence. In ECD and ETD, fragments retain post-translational modification such as phosphorylation [1-3], while a conventional low energy CAD/CID of phosphopeptides typically leads to dissociation of a phosphorylation modification during fragmentation. However, the "On-Resonance " CID method was developed mainly to reduce neutral loss and to improve sequence coverage [4]. In this study, the fragmentation of two different lengths of protonated phosphopeptides (FQpSEEQQQTEDELQDK and pYYRK) in both "On-Resonance " and "Off-Resonance " pulsed gas introduction CID were investigated using *ab initio* MO calculations.

Methods

Optimized geometries and energies of all possible conformers of proton adducts of phosphoptides of FQpSEEQQQTEDELQDK and pYYRK, reaction intermediates, transition states and fragments were calculated using *ab initio* MO methods. "On-Resonance " and "Off-Resonance " CID of the protonated phosphopeptides (FQpSEEQQQTEDELQDK and pYYRK) were observed using MALDI Quadrupole Ion Trap Time-of-Flight Mass Spectrometer (AXIMA-Resonance, Shimadzu/Kratos, UK). Protonated pYYRK was produced using the fragmentation of Insulin receptor (1142-1153) TRDIYETDpYYRK.

Preliminary Data

In "On-Resonance " pulsed gas introduction CID of protonated FQpSEEQQQTEDELQDK, the ion intensity ratios of $[b_{15}\text{-}HPO_3]$ / b_{15}, $[y_{14}\text{-}HPO_3]$ /y_{14} and $[y_{14}\text{-}H_3PO_4]$ /y_{14} were much smaller than those in "Off-Resonance " pulsed gas introduction CID. This suggests that the kinetic energy of the precursor ions in "On-Resonance " CID increased more rapidly compared with "Off-Resonance " CID. As a result, the precursor ions excited by "On-Resonance " CID promptly received enough energy to cleave the peptide bond (amide bond). On the other hand, in "Off-Resonance " CID, vibrational excitation of the precursor ions occurred gradually through multiple collisions. In this case the excitation energy was less than the energy barrier of an amide bond cleavage.

The b_2, b_3, y_2, a_2 and a_3 ions, $[b_3\text{-}HPO_3]^+$, $[pYYR\ (H^+)\ K\text{-}HPO_3]^+$ and $[pYYR\ (H^+)\ K\text{-}98]^+$ appeared in the MS/MS spectrum of Protonated pYYRK using "Off-Resonance " pulsed gas introduction CID. On the other hand, in "On-Resonance " pulsed gas introduction CID, the $[b_3\text{-}HPO_3]^+$ peak at *m/z* 483 became much smaller or even unobservable. The b_2, y_2, a_2 and a_3 peaks also became much smaller in "On-Resonance " pulsed gas introduction CID.

In order to elucidate the fragmentation mechanism of protonated pYYRK in "On/Off-Resonance " CID, potential energy surfaces for their possible fragmentation processes were calculated at the B3LYP/6-31+G (d, p) //B3LYP/6-31G (d) level [5]. From the results of *ab initio* calculations, it was found that pYYR (H^+) K is the most stable isomer of protonated pYYRK and that the production of pYYR (H^+) (OH) (*m/z* 581) has the lowest energy barrier. An elimination of K from pYYR (H^+) K accompanied with a six-membered ring formation in the R side chain results in the production of the b_3 ion (pYYR (H^+)) (*m/z* 563).

The larger peptide has the greater number of vibrational and rotational modes than the smaller peptide. Therefore, it takes more time for the larger peptide to gain enough energy for the neutral loss of HPO_3 or H_3PO_4.

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S32-1000

10:00 – 10:20

Electron transfer dissociation of protonated disulfide linked peptides and analogs without S-S bond

Shigeo Hayakawa[1], Shinya Matsumoto[1], Michisato Toyoda[2], Yasushi Shigeri[3], Michiko Tajiri[4], Yoshinao Wada[4]

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Keywords:
High energy electron transfer dissociation (HE-ETD) , Disulfide linked peptide, N-Ca backbone cleavage, Peptide sequence.

Novel aspects:
HE-ETD using alkali metal targets provides the sequence of disulfide linked peptides regardless of the ring structure by S-S bond.

Abstract:
Introduction: Disulfide linkages in polypeptides are post-translational modifications that play a pivotal role in maintaining higher order structure and biological functions. Preferential cleavage of the disulfide bond has been reported by electron capture dissociation (ECD) and low-energy electron transfer dissociation (LE-ETD) . The determination of the position of phosphorylation and amino acid sequence of phosphopeptides by high-energy electron transfer dissociation (HE-ETD) using an alkali metal target has been reported. In the present work, HE-ETD using K and Cs targets was applied to doubly protonated disulfide linked polypeptides. The difference of the peptides with S-S bond and without S-S bond is presented, and the comparison between the spectra with HE-ETD and LE-ETD is also discussed.
Method: High energy ETD spectra were measure using a sector type tandem mass spectrometer (MS/MS) . Doubly protonated peptide ions were generated by an electrospray ionization source. The doubly-protonated ions were accelerated to a kinetic energy of 10 keV by applying an accelerating voltage of 5 kV. Mono-isotopic ions were mass selected by a JEOL JMS-HX110 double focusing mass spectrometer (MS-I) . The mass-selected precursor ions were led to a 3.7 cm long collision cell in which alkali metal vapor were filled. The singly- and doubly- charge positive ions were mass-analyzed by a spherical electrostatic analyzer of a central radius of 216 mm (MS-II) . Low-energy ETD spectra were measured by Finnigan LTQ XL (ThermoFisher Scientific) using fluoranthene anion. Vasopressin (**1**) , vasotocin (**2**) , and oxytocin (**3**) were used as received. [$Ala^{1,6}$] -vasopressin (**4**) in which cysteine residues were substituted by alanine residues was synthesized by use of a Shimadzu PSSM-8 automated peptide synthesizer.
Results and Discussion : The HE-ETD and LE-ETD spectra of the doubly-protonated peptides (≡ $[M+2H]^{2+}$) of **1**, **2**, **3**, and **4** were measured with K and Cs targets. HE-ETD spectra of [$Ala^{1,6}$] -vasopressin (**4**) which has not the SS ring structure, provided all z-type ions due to NCa bond cleavage as main fragments except z_3 whose position corresponds to the proline residue. Charge reduced ions with loss of side chains, immonium ions, and some b- and y-type ions were also observed. LE-ETD spectrum also provided similar fragment ions to the HE-ETD spectrum except lack of $z-1$ and the immonium ions. In HE-ETD spectrum of vasopressin (**1**) , all the z-type ions due to both NCa and SS or CS bond cleavage except z_3 were observed regardless of the existence of the ring structure by SS bond. Most of the y-type ions were also observed in the spectrum. HE-ETD spectrum of vasotocin (**2**) provided all the y-type ions. While HE-ETD spectra of oxytocin (**3**) did not provide all y-type ions clearly, all b-type ions were observed. This difference of the observed type of the ions can be attributed to the existence of arginine residue in **1** and **2**, and lack of the arginine residue in **3**, as the arginine residue can keep positively charge in its side chain. The dependence on the target alkali metal between Cs and K did not provide the observed peaks but slight difference only for relative intensity of the observed fragments. These observed peaks in these spectra indicated that HE-ETD using an alkali metal target can provide the complete peptide sequence of the disulfide linked peptides. In the HE-ETD spectrum of **1** and **2**, z_4-S ion formed by N-Ca and C-S bond cleavage was much larger than z_4 ion which was formed by N-Ca and SS bond cleavage. Since its position correspond to next of the cysteine residue, it is presumed that hydrogen radical induced the C-S bond cleavage preferentially.

Oral Session

Thursday, 20th September

Room A

09:00 – 11:00

Session 32: Formation and Dissociation of Peptide Radical Ions

Chair: Dominic T W Chan (The Chinese University of Hong Kong, Hong Kong SAR)

S32-1020

10:20 – 10:40

Ionization energy of gas phase proteins and its dependence on charge state and structure

Alexandre Giuliani[1], Alexksandar R Milosavljevic[2], Konrad Hinsen[1], Francis Canon[1], Laurent Nahon[1], Matthieu Refregiers[1]

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Keywords:
photon activation, tandem mass spectrometry, gas phase structure, EID, photoionization

Novel aspects:
We report for the first time the relationship between the ionization energy of a protein and its charge state and structure.

Abstract:
Mass spectrometry (MS) offers the unique ability to manipulate ions in the gas phase. In particular, the potential of mass spectrometry to perform mass-selected spectroscopy on biomolecules isolated in the gas phase is very appreciable. First, electrospray ionization is a unique method to bring into the gas phase large and fragile biological species intact. Furthermore, MS is the only technique to provide additional control on the charge state of the targeted molecule. Indeed, Hirsch et al. [1] have reported spectroscopic studies on mass selected clusters of ions, while Thissen et al. [2] took profit from the storage of the ions to produce relaxed targets in their electronic ground states We report on a systemic study of the dependence of the ionization energy as a function of the protein charge states for cytochrome C, bovine pancreatic trypsin inhibitor (BPTI) and ubiquitin.

A linear ion trap (Thermo Scientific LTQ XL) has been coupled to an ultra-violet beam line at the SOLEIL synchrotron radiation facility [3] .Water acetonitrile solutions of 5 µM cytochrome C, BPTI and ubiquitin were electrosprayed at 3 µL/min. Each charge state has been selected and submitted to 100 ms irradiation between 8 and 16 eV photon energy. At each photon energy, the abundance of the photoionization product has been measured and normalized to the total ionic current and to the photon flux. From these ion yields, the ionization energy threshold has been determined.

The ionization energies for cytochrome C, BPTI and ubiquitin have been measured for charge states ranging from 4 to 15, 4 to 8 and 4 to 9, respectively. For BPTI a linear dependency of the ionization energy with the charge state is observed. This result is in line with the outcome of previous work, in which the ionization energy of peptide smaller than 3.5 kDa had been measured by electron impact [4] . The linear dependence of the ionization energies with the charge state was interpreted as the result of the increase of the attractive Coulomb potential of the ion. For ubiquitin and cytochrome C, this model is not adequate anymore, since the dependency ceased to be monotonic and he IEs appeared to plateau for particular range of charge states. The correspondence between these features in the IE vs charge state appear similar to those observed in the collision cross section obtained from ion mobility experiments.
Our molecular dynamic simulation supports this interpretation. The ionization energy of protein is thus dependent on the gas phase conformation. We propose a model which links the ionization energy, the charge state and the radius of gyration of the protein.

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Oral Session

Thursday, 20th September

Room A

09:00 – 11:00

Session 32: Formation and Dissociation of Peptide Radical Ions

Chair: Dominic T W Chan (The Chinese University of Hong Kong, Hong Kong SAR)

S32-1040

10:40 – 11:00

Direct ECD and CID sequencing intra disulfide C-terminal loops of non-tryptic natural peptides

Tatiana Y Samgina, Yegor A Vorontsov, Konstantin V Karandashev, Albert T Lebedev

M.V.Lomonosov Moscow State University

Keywords:
natural peptides, disulfide cycle, CID, ECD, direct sequencing

Novel aspects:
New fragmentation pathways of protonated molecules of natural peptides allowing for the elucidation of the sequence inside C-terminal disulfide cycle were observed in CID and ECD modes without preliminary derivatization

Abstract:
Usually mass spectrometric sequencing of peptides with an intra molecular disulfide cycle involves preliminary modification of S-S bond. The proposed earlier methods of direct sequencing of such peptides have some restrictions dealing with length and structure as well as position of S-S bond in the backbone of the molecules. The present study demonstrates ability of CID and ECD to carry on sequencing inside disulfide C-terminal loops of non-tryptic natural peptides from the skin secretion of ranid frogs without any derivatization.

The skin secretions of *Rana temporaria, Rana ridibunda, Rana esculenta and Rana arvalis* were obtained by mild electric stimulation. The skin secretion was filtrated (PTF filter, 0,45 μ), concentrated, liophylized, and analyzed with ESI LC-MS/MS. NanoHPLC-FTICR-MS system (Thermo LTQ-FT Ultra with Agilent 1100 equipped with homepacked nanocolumn) was used to record CID and ECD spectra.

CID and ECD mass spectra of 11 non-tryptic peptides containing from 15 to 35 amino acids, with C-terminal cysteine cycle were processed manually. ECD spectra of all these peptides demonstrate a peak of an ion formed due to the loss of a sulfur atom from the protonated molecules. Another group of ions represents N-C α cleavages inside S-S cycle. C-terminal fragmentation is significantly enhanced when one or better two C-terminal Lys are present. In this case 5 outof 6 possible cleavages inside 7-member "Rana box " cycle take place. An increase of the precursor ion charge state does not influence on c-*z*-ion series, although favors formation of y and *b* ions, i.e. promoting additional fragmentation pathways. Sequence coverage in ECD spectra is quite high, reaching 80 % for brevinin 1 and 88 % for brevinin 1 Vb. It is worth mentioning that the cleavage at N-terminus of C-terminal Cys was not detected for any of the studied peptides.

Despite of great number of publications on ESI CID fragmentation of polypeptides a fragmentation pathway inside C-terminal cycle of natural peptides not reported earlier was observed in the present work. The exact mass of the neutral loss in conditions of low energy CID corresponds to the cleavage of amide bond X-Cys-OH, where X is the second amino acid residue from the C-terminus. The ions peaks due to the cleavages of amide bonds at the C-terminus are present in CID spectra. This series of ions breaks with the elimination of S-sulfanylcystein (- 152.92 amu) and formation of dehydroalanine moiety (69.02 amu) at the C-terminus of the corresponding fragment ion. Thus amide bond cleavage inside the cycle rather than S-S bond cleavage takes place in conditions of ESI CID. For the Pro-containing peptides similar opening of the cycle is observed as part of secondary fragmentation processes involving primary ions forming due to the cleavage at the N-terminus of Pro. This type of fragmentation characterizes 9 peptides out of 11 studied. This direction is not a dominant one being strongly dependent on the composition of C-terminal cycle and charge state of the molecular ion. Thus the presence of basic Lys (better 2 Lys) in the loop favors this pathway significantly. For three natural peptides with 17 amino acid residues CID spectra allowed their complete sequencing.

The observed feature of ECD and CID spectra dealing with C-terminal disulfide cycles gives a chance to increase efficiency of direct sequencing of natural peptides of this type.

Oral Session

Thursday, 20th September

Room B-1

09:00 – 11:00

Session 33: JMS Award Symposium

Chair: Richard M Caprioli (Vanderbilt University,Nashville,TN,USA / Editor-in-Chief, Journal of Mass Spectrometry)

S33-0900 JMS Award -Overview and Award Ceremony-

09:00 – 09:20

Richard M Caprioli[1, 2]

[1]Vanderbilt University, Nashville, TN, USA, [2]Editor-in-Chief, Journal of Mass Spectrometry

Oral Session

Thursday, 20th September

Room B-1

09:00 – 11:00

Session 33: JMS Award Symposium

Chair: Richard M Caprioli (Vanderbilt University,Nashville,TN,USA / Editor-in-Chief, Journal of Mass Spectrometry)

S33-0920

09:20 – 09:40

Integral Membrane Proteins by High-mass MALDI-MS: Direct Access to the Stoichiometry of their Complexes and to Posttranslational Modifications

FAN CHEN[1], Sabina Gerber[2], Katrin Heuser[2], Volodymyr Kokhov[2], Christian Lizak[2], Kaspar Locher[2], Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland, [2]Institute of Molecular Biology and Biophysics, ETH Zurich, Swizterland

Keywords:
Integral Membrane Proteins (Complexes), MALDI

Novel aspects:
High-mass MALDI-MS provides a robust, detergent-friendly and straightforward access to integral membrane proteins, including the stoichiometry and posttranslational modifications, with high mass accuracy.

Abstract:
A precise understanding of the chemistry of integral membrane proteins is important for optimizing crystallization conditions and for subsequent structure determination. Mass spectrometry (MS) is a powerful and versatile tool that provides deep insight into the state of integral membrane proteins, e.g., with respect to posttranslational modification. There are, however, only very few examples where electrospray ionization (ESI) - after quite a lot of efforts for optimizing buffer and instrument conditions - or matrix-assisted laser desorption/ionization (MALDI) -MS have been used to study integral membrane proteins or their complexes. In all of these cases, the experimental conditions were highly specific and could not be easily transferred to other membrane proteins. In other words, it is still challenging for soft ionization MS to analyze integral membrane proteins and their complexes.

In this work, we succeeded in using the traditional dried droplet method, sinapinic acid as the matrix, and a commercial high-mass detector (HM2, CovalX) retrofitted to our MALDI-MS instrument to explore integral membrane proteins in detergent micelles directly. Oligosaccharyltransferase (PglB) was measured with a mass error around 0.1 %. By protecting the membrane protein complexes via chemical cross-linking with glutaraldehyde, we unambiguously determined the subunit stoichiometry of a series of ATP-binding cassette (ABC) transporter complexes, the homomeric Campylobacter jejuni-encoded ABC transporter PglK, and the heteromeric vitamin B12 importers BtuCD and BtuCDF. Moreover, the precise molecular weight determined by high-mass MALDI-MS allowed determining the site of N-linked glycosylation in Candida aibicans drug resistance protein 1 (Cdr 1 p), which assists in circumventing problems due to heterogeneous glycosylation in future crystallization studies. All the above information would not be accessible at all with SDS-PAGE or gel filtration chromatography, the commonly used tools in laboratories studying membrane proteins.

Oral Session

Thursday, 20th September

Room B-1

09:00 – 11:00

Session 33: JMS Award Symposium

Chair: Richard M Caprioli (Vanderbilt University,Nashville,TN,USA / Editor-in-Chief, Journal of Mass Spectrometry)

S33-0940

09:40 – 10:00

Great Insights from a Small System: Structure and reactivity of [VPO4]+ in comparison with [V2O4]+.

Nicolas Dietl, Maria Schlangen, Helmut Schwarz

Technical University Berlin, Berlin, Germany

Keywords:
oxo-clusters, DFT-calculations, IRPD-spectroscopy, gas-phase reactions

Novel aspects:
Improved mechanistic understanding of structure-reactivity relationships in thermal reactions of oxo-clusters with small hydrocarbons in the gas phase.

Abstract:
The selective oxidation of hydrocarbons still constitutes one of the major challenges in contemporary chemistry to solve global problems, such as the environmentally benign and economically feasible conversion of natural gas into value-added products.[1] Today, there exist numerous effective homogeneous and heterogeneous catalysts which cover a broad spectrum of oxidation reactivity ; however, it is not exaggerated to note the lack of substantiated knowledge about the intrinsic properties of many of the catalysts which, after all, control the selectivity of the various oxidation processes. A well-known example for such a poorly understood catalytic process represents the chemical transformation of *n*-butane to maleic anhydride by the so-called *VPO*-catalysts ; this highly complex and selective reaction involves the abstraction of eight hydrogen atoms from, as well as the transfer of three oxygen atoms to C_4H_{10}. One approach in the elucidation of mechanistic aspects at a molecular level employs reactivity studies which are conducted under near single-collision conditions in a mass spectrometer in conjunction with computational studies.[2]
Our recent results report on the electrospray-ionization (ESI) generation of the small, mixed-oxo cluster $[VPO_4]^+$, its electronic structure in the gas phase as well as its reactivity towards small hydrocarbons, thus permitting a comparison to the long-time known and extensively investigated vanadium-oxide cation $[V_2O_4]^+$.[3] As described in previous studies, the latter exhibits no reactivity towards small hydrocarbons, such as CH_4, C_2H_6, C_3H_8, C_4H_{10}, and C_2H_4, while the substitution of one vanadium atom by a phosphorous atom yields the reactive $[VPO_4]^+$ ion which brings about oxidative dehydrogenation from saturated hydrocarbons, i.e. propane and butane, as well as oxygen-atom transfer to unsaturated hydrocarbons, i.e. ethene, at thermal conditions. Further, the structure of $[VPO_4]^+$ was characterized in the gas phase by advanced IR photodissociation spectroscopy, and a comparison is made to the also structurally characterized gaseous $[V_2O_4]^+$ system, including structure-reactivity relationships. Finally, reaction mechanisms are elucidated by DFT calculations ; the results underline the key role of phosphorous in terms of C-H bond activation of hydrocarbons by mixed *VPO*-clusters.

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S33-1000
10:00 – 10:20

Charge-State Dependent Compaction and Dissociation of Protein Complexes: Insights from Ion Mobility and Molecular Dynamics

Zoe Hall[1], Argyris Politis[1], Matthew F Bush[1,2], Lorna J Smith[1], Carol V Robinson[1]

[1]University of Oxford, Oxford, UK, [2]University of Washington, Seattle, USA

Keywords:
ion mobility CID protein complex

Novel aspects:
The charge-state dependece of protein complex CID pathways is demonstrated by IM-MS, in combination with charge manipulation in solution, and is supported by solvent-free molecular dynamics simulations.

Abstract:
Combining computational methods with gas-phase experiments can provide powerful insights into the structure and interactions of biomolecular ions. Here we combine ion mobility-mass spectrometry (IM-MS) with molecular dynamics (MD) simulations to investigate the structural changes occurring in four multimeric protein complexes during gas-phase activation[1]. Importantly, using IM-MS, we find that all four complexes retain their native-like topologies at low energy. Upon increasing the collision energy, two of the four complexes adopt a more compact state. This collapse was most noticeable for pentameric serum amyloid P (SAP) which contains a large central cavity. The extent of collapse was found to be highly correlated with charge state, with the surprising observation that the lowest charge states were those which experience the greatest degree of compaction. Intermediate charge states unfolded without prior collapse, releasing highly charged, extended monomers. The highest charge states ejected compact monomers and dimers. We compared our experimental results with *in vacuo* MD simulations of SAP, which were carried out over increasing temperature, in order to impart increasing amounts of energy to the system, to mimic IM-MS activation experiments. Simulations showed that low charge states of SAP exhibited compact states, corresponding to collapse of the ring, whilst intermediate and high charge states unfolded to more extended structures, maintaining their ring-like topology, as observed experimentally. To simulate the collision-induced dissociation (CID) of different charge states of SAP, we used MS to measure the charge state of the ejected monomer and assigned this charge to one subunit, distributing the residual charges evenly among the remaining four subunits. Under these conditions, intermediate charge states of SAP dissociated *via* the unfolding and ejection of a single subunit. The highest charge states dissociated to compact monomers and dimers with similar collision cross sections to those released in CID experiments for supercharged SAP. This strong correlation between theory and experiment has implications for further studies as well as for understanding the process of CID and for applications to gas-phase structural biology more generally.

References

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Oral Session

Thursday, 20th September

Room B-1

09:00 – 11:00

Session 33: JMS Award Symposium

Chair: Richard M Caprioli (Vanderbilt University,Nashville,TN,USA / Editor-in-Chief, Journal of Mass Spectrometry)

S33-1020
10:20 – 10:40

Odd products from even-electron ions: Exploiting charge-remote bond homolysis to compare bond dissociation energies

David L Marshall[1], Martin Paine[1], Philip J Barker[2], Stephen J Blanksby[1]

[1]University of Wollongong, Wollongong, Australia, [2]BlueScope Steel Research, Port Kembla, Australia

Keywords:
Nitroxyl radical, alkoxyamine, even-electron rule

Novel aspects:
CID of alkoxyamines produces structure-dependant competitive bond homolysis, violating the even-electron rule. Relative BDEs are derived in agreement with theoretical calculations.

Abstract:
Collision induced dissociation (CID) of even-electron precursor ions typically yield even-electron fragment ions : a phenomenon known as the "even-electron rule ". There are, however, exceptions where even-electron ions fragment via bond homolysis, particularly where the result is a stable radical ion. When independent of the charged moiety, the efficacy of homolysis is indicative of the bond dissociation energy (BDE) . In this research we derive the relative BDEs of N-O and O-C bonds in alkoxyamines to provide new insight into the mechanistic action of these anti-oxidants.
Hindered amine light stabilisers are commonly added to polymer coatings. The key to their anti-oxidant action involves nitroxides ($R^1R^2NO^{\cdot}$) scavenging macroradicals to form alkoxyamines ($R^1R^2NOR^3$) , thus inhibiting radical induced degradation. There exists significant debate about the fate of the alkoxyamine and the mechanism (s) by which the nitroxyl radical is recycled. Mass spectrometric analyses of stabilised polyesters exposed to high temperatures identified both secondary amine (R^1R^2NH) and alkoxyamine products. [1] One plausible explanation is that N-O homolysis competes with O-C homolysis, the former resulting in an aminyl radical and ultimately a secondary amine.
To test this hypothesis, a suite of alkoxyamines were synthesised, each incorporating a remote acidic moiety, but differing in the nature of the R^3 functional group. The $[M\text{-}H]^-$ ions formed from electrospray ionisation of these alkoxyamines were subsequently activated by collision induced dissociation in a triple quadrupole mass spectrometer.
The fragmentation of alkoxyamine precursor ions to nitroxides by O-C homolysis is shown to be another violation of the "even-electron rule. " This is not surprising, given the inherent stability of nitroxides. Transient aminyl radicals are also identified, from a competitive N-O homolysis channel, which for certain R^3 functionalities dominates O-C homolysis. The relative efficacy of these processes can be attributed to the relative stability of the alkyl and alkoxyl radicals. Remarkably, no significant even-electron product channels are observed upon CID. The selective formation of nitroxyl and aminyl radicals, together with the variable energy imparted in the triple quadrupole, has been used to probe the structure-dependant relative thresholds of N-O and O-C bond homolysis. Whilst this technique does not provide the precision of guided ion beam methods, the qualitative trends are supported by ab initio quantum chemical calculations. [2]
Understanding the structure-dependant relationship between these competing processes will assist in the selection of systems that selectively promote one homolysis pathway. Furthermore, this distonic ion approach may prove useful for the further characterisation of aminyl radicals in the gas phase, or the evaluation of polymer stabilisation mechanisms by alkoxyamines.

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Oral Session

Thursday, 20th September

Room D

09:00 – 11:00

Session 34: MS Informatics for Identification and Characterization

Chair: Shigeki Kajihara (Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Co , Japan)

S34-0900

09:00 – 09:40

[Keynote Lecture] The value of different types of information in MS based identification

David Fenyö

New York University, New York, NY, USA

Keywords:
MS Informatics

Novel aspects:
The value of different types of information in MS based identification

Abstract:
Mass spectrometry-based protein identification has become an invaluable tool for elucidating protein function, and several methods have been developed for protein identification, including sequence collection searching with masses of peptides and their fragments, spectral library searching, and de novo sequencing. In all mass spectrometry-based identification methods, a score is calculated to quantify the match between the observed mass spectrum and the collection of possible sequences. These scores are highly dependent on the details of the algorithm used, and they are not always easy to interpret because the interpretation of the score depends on properties of the data and the search results. Therefore, it is desirable to convert the score to a measure that is easy to interpret, such as the probability that the result is random and false. It is also important to choose an appropriate collection of sequences to search, because if the search space is expanded to include a lot of irrelevant sequences, the sensitivity decreases, but if a sequence is not included it will not be found. The current development of next-generation sequencing technologies has made it possible to within the budget of many proteomics projects to also generate RNA-Seq data, i.e. sequence the transcriptome, and use it to construct sample-specific sequence collections for searching. Here, we will give an overview of mass spectrometry-based protein identification and discuss strengths and weaknesses of different strategies.

Oral Session

Thursday, 20th September

Room D

09:00 – 11:00

Session 34: MS Informatics for Identification and Characterization

Chair: Shigeki Kajihara (Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Co , Japan)

S34-0940

09:40 – 10:00

Improving endogenous peptide sequence characterization using electron-capture dissociation and collision-induced dissociation.

Eisuke Hayakawa[1], Gerben Menschaert[2], Walter Luyten[1], Geert Baggerman[3], Liliane Schoofs[1]

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Keywords:
peptide identification, peptidomics, ECD, ETD, MS/MS

Novel aspects:
A combination of two fragmentation methods, CID and ECD, enables highly accurate and confident endogenous peptide identification. This approach is also effective for peptide identification in large search space.

Abstract:
Tandem mass spectrometry (MS/MS) and bioinformatics tools have enabled fast and systematic peptide identification. Especially, the combination of LC-MS/MS and MS/MS peptide search software has become the method of choice for the characterization of peptides in complex biological samples. However, it is still a challenge to achieve accurate characterization of endogenous peptides, such as neuropeptides, peptide hormones, peptide pheromones and antimicrobial peptides, at high confidence from MS/MS spectra. Since various types of enzymes are potentially involved in the processing of endogenous peptides, the MS/MS search has to be done without any cleavage site specification. In addition, many endogenous peptides have various post-translational modifications (PTMs) and these need to be taken into account in the search. These characteristics of endogenous peptide lead to a huge search space and result in poor confidence of peptide characterization. As a consequence, characterization of endogenous peptide from MS/MS spectra is still difficult.

We developed a new MS/MS peptide search method to enable highly accurate and confident endogenous peptide characterization by using a combination of two different fragmentation methods. Collision-induced dissociation (CID) is commonly used as a peptide fragmentation method in general LC-MS/MS analysis. On the other hand, relatively new methods : electron-capture dissociation (ECD) or electron-transfer dissociation (ETD) , achieve fragmentation that is complementary to CID. We have employed a new approach to take advantage of the combination of these two independent fragmentation methods for highly accurate endogenous peptide identification. Peptide-spectrum matching (PSM) is carried out for CID and ECD separately, and the score of a peptide sequence is given as a combination of the two PSM scores. This approach is quite effective to discriminate correct peptide characterization from false hits and therefore enables highly accurate and confident peptide identification. We applied this new approach to CID and ECD spectra of mouse neuropeptides for validation. This method significantly increased the peptide identification rate and the reliability, as compared to a conventional MS/MS peptide search using a single fragmentation method. Especially, it is effective for endogenous peptide identification with various post-translational modifications against large protein databases.

This new MS/MS search method will contribute to the accurate and confident identification of novel endogenous peptides. Furthermore, this method will be effective for other MS/MS peptide search applications that require to be done against large databases, such as proteogenomics, peptidogenomics and metaproteomics.

Oral Session

Thursday, 20th September

Room D

09:00 – 11:00

Session 34: MS Informatics for Identification and Characterization

Chair: Shigeki Kajihara (Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Co , Japan)

S34-1000

10:00 – 10:20

LipidXplorer software supports high-throughput shotgun lipidomics at any mass spectrometer platform and acquisition mode

Ronny Herzog[1], Dominik Schwudke[2], Kai Schuhmann[1], Julio Sampaio[1], Andrej Shevchenko[1]

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Keywords:
Lipidomics, Computational, Mass Spectrometry, High Throughput

Novel aspects:
LipidXplorer software supports high-throughput shotgun lipidomics at any mass spectrometer platform and acquisition mode.

Abstract:
Lipidomics aims at quantifying the full lipid complement in cells, tissues or organisms. Shotgun lipidomics relies on the direct analysis of total lipid extracts, in which lipids are recognized by either accurately determined *m/z*in survey MS spectra or by structure-specific "signature ions " in MS/MS spectra of prospective precursors. The shotgun approach has been widely applied in biological research, but a major bottleneck still exists in the accurate interpretation of spectra. The available software is typically tuned to a particular type of mass spectrometer, spectra acquisition and lipid identification method.

Here we report on LipidXplorer that supports shotgun lipidomics by any common acquisition method at any instrumentation platform. Importantly, it offers resolution-dependent interpretation of MS and MS/MS spectra and supports high throughput lipidomics screens. The interpretation of shotgun MS and MS/MS spectra relies upon the Molecular Fragmentation Query Language (MFQL) [1] and is independent from reference spectra libraries or lipidomics databases. Using MFQL, any lipid identification routine is described in simple and intuitive terms and can be adjusted to particular instrumentation features (mass accuracy and mass resolution) and instrument-dependent fragmentation pathways. In particular, common MFQLs support identification and quantification of lipids on triple quadrupole, ion trap, hybrid quadrupole time-of-flight and linear ion trap Orbitrap instruments by multiple reaction monitoring, data-dependent MS/MS, precursor and neutral loss scans and accurate mapping of intact masses [2] . We report on benchmarking LipidXplorer lipid identification performance and validated its cross-platform capabilities by comparative analysis of total lipid extracts on different mass spectrometric platforms.

LipidXplorer has its own wiki page that features MFQL queries library linked to more than 14 application papers. It also provides a full documentation, step-by-step tutorial and test example queries and datasets.

Since tandem mass spectrometers are now commonly available, we argue that LipidXplorer eliminates one of the major hurdles in adopting lipidomics methodologies by a broad scientific community in the fields of cell biology and molecular medicine.

References：

[1] R. Herzog, D. Schwudke, K. Schuhmann, S. Bornstein, M. Schroeder and A. Shevchenko. A Novel Informatics Concept for High-Throughput Shotgun Lipidomics Based on the Molecular Fragmentation Query Language

[2] R. Herzog, K. Schuhmann, D. Schwudke, J. L. Sampaio, S. R. Bornstein, M. Schroeder, A. Shevchenko. LipidXplorer：A Software for Consensual Cross-Platform Lipidomics PLoS ONE 7 (1)：e29851. doi：10.1371/journal.pone.0029851.

Oral Session

Thursday, 20th September

Room D

09:00 – 11:00

Session 34: MS Informatics for Identification and Characterization

Chair: Shigeki Kajihara (Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Co , Japan)

S34-1020 MassBank: Public Mass Spectral Database

10:20 – 10:40

Takaaki Nishioka, Yoshito Nihei, Yuya Ojima, Tasuku Ikeda
Nara Institute of Science and Technology, Nara, Japan

Keywords:
peak annotation, structure elucidation, public repository

Novel aspects:
Chemical annotation of ESI-MS 2 data of high mass accuracy are useful for the analysis of peak-chemical structure relationships. These were applied to the chemical structure elucidation of unknown ESI-MS 2 data.

Abstract:
[**Summary**] Mass spectral data, as the experimental data supporting research conclusions, should be open to the public for allowing other researchers to verify the experiments. Additionally they are valuable research products that should be shared among the researchers using mass spectrometry for chemical identification and other applications. MassBank (http : //www.massbank.jp) has been internationally accepted as a public repository of mass spectra of small chemical compounds. ESI-MS^2 data of high mass accuracy have steadily increased in MassBank. Their product ions are so accurately analyzed that we have successfully annotated with the molecular formula. MassBank provides users with these data that are searchable by molecular formulae as well as conventional peak *m/z* values. We report novel applications of the chemically annotated ESI-MS^2 data in MassBank to the structure elucidation from LC-MS^2 data.

[**Current Data Statistics**] Researchers have made their data open to the public from MassBank at their own expenses. That is, they have to prepare their records in the MassBank Record Format and provide their MassBank data servers. In spite of such expenses, MassBank contributors and their data increase steadily. Currently (April 2012) , 21 research groups (15 Japan, 3 USA, 2 Germany, 1 China) deposit a total of 29,644 high-quality MS data of 13,534 small molecules, which include ESI-MS, MS^2 16,440 data of 2,204 compounds from nine data servers. Among them, the number of ESI-MS^2 data of relative mass accuracy within 50 ppm is 6,146. Researchers timely share the data analyzed on up-to-date instrument models. Accesses to MassBank in the period of October, 2011 were 10,063 unique IP addresses per month.

[**Tools for Sharing Mass Spectral Data**] Mass++ (http : //www.first-ms 3 d.jp/english/achievement/software) and MassBank projects have collaborated to relieve the data contributors and users of tedious manual tasks in data preparations and retrieves. For example, in metabolomics study, a single LC-MS^2 analysis of a biological sample outputs hundreds of MS^2 data. With these outputs as queries, spectral search of the MassBank data for the metabolite identification was a tedious manual work previously. The two projects developed the batch spectral search, by which users are able to submit a set of the query data at once.

[**Chemical Annotation of MassBank ESI-MS^2 Data**] We manually annotated the molecular formula to product ions observed in the MassBank ESI-MS^2 data that were analyzed on ESI-QTOF and ESI-QFT mass spectrometers. The annotated spectral data are searchable with the molecular formula of peaks and peak differences on MassBank. In combination with the substructure search, peak search with the molecular formula is helpful to analyze the relationships between product ions and chemical substructures and to evaluate the specificity of the relationships. These relationships are accumulated as Fragmentation Library on a wiki system (http : //metabolomics.jp/wiki/Index : MassBank) .

One of the applications of the relationships is the chemical structure elucidation of unknown metabolites from their ESI-MS^2 data. The relationships and KNApSAcK database (http : //kanaya.aist-nara.ac.jp/KNApSAcK/) are integrated in a MassBank tool that predicts the chemical substructure of unknown metabolites. Currently the tool works to predict unknowns that are derivatives of primary metabolites. To predict secondary metabolites more effectively, the relationships should cover broader substructures that secondary metabolites integrate. Identification or structure elucidation of unknown metabolites is a bottleneck of metabolomics study. Sharing mass spectral data of secondary metabolites is a steady and effective solution to remove the bottleneck.

[**Acknowledgements**] The authors thank to all the researchers who contribute MassBank with their data at their own expenses. MassBank is the official database of the Mass Spectrometry Society of Japan. MassBank project is financially supported by "Program for Coordination Toward Integration of Related Databases ", the National Bioscience Database Center in Japan.

Oral Session

Thursday, 20th September

Room D

09:00 – 11:00

Session 34: MS Informatics for Identification and Characterization

Chair: Shigeki Kajihara (Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Co , Japan)

S34-1040

10:40 – 11:00

MSPTM-DB: a known PTM database for high-speed and accurate search available on the "ProteoAnalysis" web site

Akiyasu C Yoshizawa[1], Tsuyoshi Tabata[2], Takayuki Kimura[2], Ken Aoshima[2], Yoshiya Oda[2], Shigeki Kajihara[1], Koichi Tanaka[1]

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Keywords:
database, post-translational modification (PTM) , sequence, literature, annotation

Novel aspects:
We constructed two databases : One contains variety of peptide sequences with known PTM sites for high-speed and accurate search. The other contains literature information about these PTMs for rich annotation.

Abstract:

Introduction

The analysis of mass spectrometry-derived data is the mainstream of proteomic research, although software for this purpose is still evolving. We are constructing a web site for proteomic research called "ProteoAnalysis, " and implementing several new functions for the analysis of mass spectrometric data. Using functions on this site, users can extract peak lists from raw data, and identify the proteins corresponding to the MS peaks : During this process, users can utilize our original databases, "MSPTM-DB " and "PTM annotation DB. " MSPTM-DB is an attempt at high-speed and accurate search for sequences with *known* (database-recorded) post-translational modifications (PTMs) , whereas the *conventional variable modification method* calculates all possible PTMs. PTM annotation DB is a database of detailed information of known PTMs. In addition to this, the sequence databases of isoforms and mutated proteins are also available on ProteoAnalysis. Using these databases, users can obtain more accurate and rich information of PTMs in a shorter time.

Methods and Materials

For the construction of MSPTM-DB, we extracted data from the "Modified Residue " lines of the "Feature " section in the Swiss-Prot database (Release 2012_04) . At this time, we focus on the phosphorylation and acetylation data ; we adopted the phosphorylation information of Serine (S) , Threonine (T) , and Tyrosine (Y) , and the acetylation information of Lysine (K) , Alanine (A) , and Methionine (M) . These data correspond to 98.8 % and 90.2 % of data in each category, respectively, and collectively cover 85.3% of all PTM data in Swiss-Prot.

We produced trypsin-digested peptides of all extracted sequences mentioned above ; we made a variety of peptide sequences with one or more known modified sites, whose known (potential) PTM sites are either modified or not modified. In the database search, characters not assigned to any amino acid are treated as "new amino acids " by temporally assigning them to the modified amino acid residues (We call this technique "*Stand-in method* ") . If "ProteoAnalysis " users just specify this database as the search database, all processes including the *Stand-in method* are automatically executed.

"PTM annotation DB " at this time contains the literature information of all PTMs recorded in Swiss-Prot. Users can look up literature information by inputting the protein name, the position of the modified amino acid, and the kind of PTM as the search keys.

To assess the usability, we performed the database search of HeLa cell extract, which was reduced, alkylated, and digested by Lys-C and Trypsin, and phosphopeptides were enriched by immobilized metal affinity chromatography (IMAC) . The search engine was X! Tandem Version : 2010.01.01.4. The search results against our *MSPTM-DB*, isoform/mutation database, and Swiss-Prot were compared with the results obtained by *the conventional search* against our isoform/mutation database and Swiss-Prot. For this evaluation test, we chose targeted PTM data according to the irreliabilities ; we used experimentally confirmed data only.

Results

Using *MSPTM-DB* for the phosphorylation analysis, we could identify **367** peptides (both phosphorylated and non-phosphorylated) in contrast with that **321** peptides were identified by *the conventional method* and **249** peptides were identified by *the normal search method*. Although *theMSPTM-DB method* cannot detect unrecorded PTMs, it can however detect both unmodified peptides and peptides with known PTMs ; surprisingly, it managed to detect peptides that were not detected by *the conventional method*. In the case of acetylated peptides, the effect of MSPTM-DB is larger ; **more than 100** additional peptides were detected compared with *the conventional method*. The search speed using *the MSPTM-DB method* is at least **20** times faster.

We are expanding the data contents and resources of both databases ; and the “ProteoAnalysis “ web site will be freely accessible on the web in the near future.

Oral Session

Thursday, 20th September

Room E

09:00 – 11:00

Session 35: Environment I

Chair: Peter Haglund (Umeå University, Sweden)

S35-0900

09:00 – 09:40

[Keynote Lecture] Chiral Chemicals as Tracers of Sources and Fate Processes in a World of Changing Climate

Terry F Bidleman[1,2,3], Liisa M Jantunen[2], Perihan B Kurt-Karakus[4], Fiona Wong[5]

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Keywords:
persistent organic pollutants, pesticides, chiral, climate, arctic

Novel aspects:
Chiral compounds offer special advantage for following POPs pathways because of their ability to distinguish emissions from racemic (newly released or protected from microbial attack) and nonracemic (microbially weathered) sources.

Abstract:
Elimination of persistent organic pollutants (POPs) under national and international regulations reduces "primary " emissions, but "secondary " emissions continue from residues deposited in soil, water, ice and vegetation during former years of high usage. In a future, secondary source controlled world, POPs follow the cycle of organic carbon and biogeochemical processes determine their transport, accumulation and fate (1). Climate change is likely to affect mobilisation of POPs through e.g., increased temperature, loss of ice cover in polar regions, melting glaciers and changes in soil microbiology which affect degradation and transformation (2). Chiral compounds offer special advantage for following transport and fate pathways because of their ability to distinguish racemic (newly released or protected from microbial attack) and nonracemic (microbially weathered) sources. Use of GC or LC with chiral stationary phases and MS makes enantiomer-specific analysis possible for chiral compounds of many chemical classes. This paper discusses the rationale for this approach and suggests applications where chiral POPs (3) and other compounds could aid investigation of climate-mediated exchange and degradation processes. Examples include distinguishing agricultural vs. non-agricultural and recently used vs. residual pesticides, degradation and sequestration processes in soil, historical vs. recent atmospheric deposition, sources in arctic air and influence of ice cover on volatilisation.

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Oral Session

Thursday, 20th September

Room E

09:00 – 11:00

Session 35: Environment I

Chair: Peter Haglund (Umeå University, Sweden)

S35-0940 POLLUTION OF MOSCOW AIR: GC/MS STUDY OF SNOW SAMPLES

09:40 – 10:00

Olga V Polyakova[1], Viatcheslav V Artaev[2], Dmitry M Mazur[1], Albert T Lebedev[1]

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Keywords:
organic pollutants, GC/MS, ICP/MS, high resolution mass spectrometry, snow

Novel aspects:
A representative list of organic pollutants in the atmosphere of Moscow was created for the first time using high resolution mass spectrometry.

Abstract:
Moscow is the largest European city with population about 15 millions and hundreds of enterprises. More than 4 millions cars are registered in Moscow. However just the most common atmospheric pollutants are monitored in the city at the regular basis. To propose a list of priority pollutants for the atmosphere of Moscow 16 snow samples were collected along the perimeter (109 km) of the Moscow belt road at the end of March 2011 and 2012. Snow is an excellent preserving matrix allowing keeping the majority of chemical compounds including not very stable ones (e.g. phenols) . In the countries with cold climate analysis of snow gives a chance to estimate long term atmospheric pollution (several months) .
Mass spectrometry was used as an analytical tool to identify individual organic compounds (gas chromatography/mass spectrometry, GC/MS) and the most environmentally relevant chemical elements (inductively coupled plasma with mass spectrometric detection, ICP-MS) . Sample preparation was carried out according to US EPA 8270 and 200.8 Methods, while LECO Pegasus IVD and Agilent7500c instruments were used correspondingly. Both target and non-target approaches were used. As a result more than 500 organic compounds belonging to various classes were identified in each sample. Besides classic pollutants like PAH, PCB, phthalates several classes of other anthropogenic contaminants including, organophosphates, esters of N,N-diethylcarbamodithioic acid, various nitrogen and sulphur containing compounds, antioxidants, and some others were represented by a number of compounds. Quite surprising was the detection of several compounds with dichloromethyl group in 2012 samples, including dichloronitromethane as the major ingredient. The confirmation of the identification was obtained by parallel analysis of all the samples with LECO Pegasus GC-HRT instrument with the resolving power exceeding 60000. Several valuable issues concerning reliability and new possibilities for the identification of new compounds were discovered when dealing with a high resolution instrument.
The levels of organic compounds using internal standards as well as the levels of chemical elements were quantified. The data obtained allow estimating atmospheric pollution in Moscow in the period between December and March and proposing a draft list of priority pollutants for the atmosphere of Moscow.

Oral Session

Thursday, 20th September

Room E

09:00 – 11:00

Session 35: Environment I

Chair: Peter Haglund (Umeå University, Sweden)

S35-1000

10:00 – 10:20

AQUEOUS-PHASE REACTIONS OF ATMOSPHERICALLY RELEVANT VOLATILE ORGANIC COMPOUNDS THROUGH TANDEM MASS SPECTROMETRY: AN INTRIGUING STORY OF AEROSOL FORMATION

Rafal Szmigielski, Krzysztof Jan Rudzinski, Inna Kuznietsova

Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland

Keywords:
volatile organic compounds, isoprenoids and their oxygenated metabolites, atmospheric aerosols, tandem mass spectrometry

Novel aspects:
The presented study falls into a hot topic research on the formation, transformation and environmental implications of ambient aerosols (SOA). Our research revealed a number of novel wet aerosol components.

Abstract:
Volatile organic compounds (VOC) are a broad group of species that play important roles in the environment. They come to existence in the air through different pathways, ranging from the vital activity of plant vegetation (a prevailing source) to anthropogenic emissions (a minor source). Regardless of the emission source, they are engaged in the formation of ambient secondary organic aerosols (SOA) in the atmosphere through a complex photo-oxidation chains followed by a gas-to-particle conversion. In addition, they affect human health, a quality of life, and in the larger scale the Earth's climate. A poorly understood chemical transformations of VOCs contribute to a significant fraction of organic matter in the atmosphere (~35% to ~90%) which is "frozen" in the form of aerosol particles.

Isoprene (2-methyl-buta-1,3-diene) and its first oxidation products (i.e., methacrolein, methyl vinyl ketone and methacrylic acid) are relevant volatile precursors of ambient SOA in the atmosphere. Isoprene is the most abundant non-methane hydrocarbon emitted to the atmosphere as a result of living vegetation. According to the recent data, the isoprene emission rate is estimated to be at the level of 700 TgC per year. While heterogeneous transformations of isoprene and its first oxidation products have been well documented, aqueous-phase reactions of these species with radical intermediates leading to the production of new class of wet SOA components such as polyols and their sulfate esters (organosulfates), are still poorly recognized. The chain reactions of isoprene with sulfoxy radical-anions (SRA) are one of the recently researched routes leading to the formation of organosulfates in the aqueous phase. The latter radical species originate from the auto-oxidation of SO_2 in the aqueous phase and are behind the phenomenon of atmospheric acid rain formation. This is a complicated chain reaction that is catalyzed by transition metal ions, such as manganese (II), iron (III) and propagated by sulfoxy radical anions.

The presented work addresses the chemical interaction of selected VOCs such as isoprene, methacrolein, methyl-vinyl ketone, with sulfoxy radical-anions in the aqueous solution under both dark and solar conditions. We showed that triple quadrupole mass spectrometry is a powerful technique to follow the chemical changes of monitored processes. The use of collision-induced dissociations revealed the formation of novel components of wet SOA, including oxygenated polar species with C-5 skeleton bearing SO_3H (MW 182, 180) and SO_2H (MW 166, 164) moieties on the hydroxyl group. The structures of these products were firmly confirmed by comparison of their liquid chromatography and mass spectrometry behaviors with that corresponding to the synthesized model compounds. It is believed that newly discovered highly polar low molecular weight compounds may contribute to the growth of wet aerosol particles by the formation of higher molecular weight species.

Oral Session

Thursday, 20th September

Room E

09:00 – 11:00

Session 35: Environment I

Chair: Peter Haglund (Umeå University, Sweden)

S35-1020

10:20 – 10:40

HRAM Screening and Quantitative Analysis of Pesticides in Environmental & Food Matrices using a bench top LCMS Orbitrap system

Dipankar Ghosh, Charles Yang, Leo Wang, Jonathan Beck

Thermo Fisher Scientific

Keywords:
Pesticides, Orbitrap, LCMS, UHPLC

Novel aspects:
Usage of a novel Second Generation Orbitrap platform to screen, quantify and confirm a large number of pesticides at the low ppb level.

Abstract:

Introduction:
Current methodologies for the quantitation of pesticides in food revolve around using triple quadrupole platforms and long run times. The method described here utilizes UHPLC- with a second generation Exactive™ mass spectrometer (MS) using high resolution accurate mass. The second generation Exactive MS is capable of resolving power settings of up to 140,000 (FWHM) at m/z 200, providing the ability to resolve matrix from analyte in full MS mode even in most complex matrices. This work describes a method to do screening and quantitation of a 100 pesticides mixture together with high level confirmation.

Method:
A Hypersil Gold aQ C18 50x2.1mm 1.9u column was utilized with a run time of less than 8 mins with all analytes eluting within 5 mins. A standard curve containing 110 compounds was spiked in orange solution ranging from 50 pg/mL levels to 250 ng/mL levels was injected in triplicate and screening of different food matrix (green bell pepper and hot peppers) samples was analyzed for targeted list of 100 compounds and also screened for possible other unknown pesticides. The spectrometer was set to a resolving power of 70,000 (FWHM) at m/z 200 in full MS mode to minimize matrix interference, and all-ion-fragmentation (AIF) spectra were collected to qualify. The data was then compared to a current MS/MS library for confirmation, as well as calibration curves were generated for the individual compounds.

Preliminary Data:
Calibration lines were generated for the compounds analyzed with r^2 better than 0.9940, the limits of detection (LOD) varied from 50pg/mL to 500pg/ml based on the individual compounds. For confirmation of each compound the exact mass of the compound, its isotopes and as well as the AIF produced were collected and compared to limit the amount of "false positives "in the results. One of the main challenges using a high resolution accurate mass system is data mining, but with novel ExactFinder™ software the data processing becomes straight forward. Using the novel second generation Exactive the workflow to screen and confirm in a single run increases throughput for repeat runs.

Oral Session

Thursday, 20th September

Room E

09:00 – 11:00

Session 35: Environment I

Chair: Peter Haglund (Umeå University, Sweden)

S35-1040

10:40 – 11:00

Development and validation of methodology using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for monitoring for use of anabolic steroids in animals

Simon J Hird, George Stubbings

The Food and Environment Research Agency

Keywords:
anabolic steroids, urine, serum, LC-MS/MS

Novel aspects:
High sensitivity, validated methodology suitable for official control purposes, chromatographic and mass spectrometric selectivity, overcoming matrix effects

Abstract:
A wide range of anabolic steroids has been used in animal fattening because of their capacity to increase weight gain and the improvements in feed conversion efficiency. The use of anabolic steroids in animal fattening is prohibited in the European Community and monitoring for use of anabolic steroids is carried out through the National Plans of the individual Member States. For controls at retail level and for products imported in the EU, it is necessary to have analytical methods applicable to meat samples, whereas at farms and abattoirs, misuse of anabolic steroids in living animals is monitored by analyses of the animal's urine and/or serum. Because of the complexity of these matrices and the low "Recommended Concentrations " established, it is necessary to have sensitive, selective and specific methods for the detection of the anabolic steroids.

Methodology has been developed for the determination of 15 anabolic steroids in bovine, ovine and porcine urine. The procedure involved enzymatic hydrolysis prior to extraction and SPE prior to analysis by ultra high performance liquid chromatography coupled to a tandem mass spectrometer operating in electrospray, switching between positive and negative ion modes. Data acquisition was performed in selected reaction monitoring (SRM) mode. The method was successfully validated according to the Commission Decision 2002/657/EC for the detection and confirmation of residues in products of animal origin. The methodology developed is suitable for the detection, quantification and confirmation of identity of these anabolic steroids in bovine, ovine and porcine urine and can be used for residue control programs.

Oral Session

Thursday, 20th September

Main Hall

15:00 – 17:00

Session 36: Advances in Ion Mobility Mass Spectrometry

Chair: Joseph A Loo (University of California-Los Angeles, USA)

S36-1500

15:00 – 15:40

[Keynote Lecture] Peptide and protein aggregation: Mechanisms, inhibition and disease

Michael T Bowers

University of California at Santa Barbara, Santa Barbara CA USA

Keywords:
ion mobility, amyloid, Alzhimer's, aggregation

Novel aspects:
IMS-MS can not only determine oligomer distributions and structures for aggregating systems but can also effectively screen small molecule inhibitors to this aggregation.

Abstract:
One of the outstanding problems in understanding amyloid diseases at the molecular level is an inability to determine oligomer distributions and oligomer structures for rapidly aggregating systems. This kind of aggregation characterizes a wide variety of important diseases including Alzheimer's disease, Type 2 Diabetes, Parkinson's disease and many others. The problem is traditional spectroscopic structural methods simply don't work since they measure average solution properties and individual oligomers cannot be isolated for x-ray analysis. In the past several years we have shown that ion mobility based mass spectrometry (IMS-MS) is ideally suited to attack this important problem. Here we will present our latest results on peptide assembly mechanisms and results on inhibition of this assembly for important disease systems.

S36-1540

15:40 – 16:00

Behavior of intrinsically disordered regions within a protein complex of Swi5-Sfr1 characterized by IM-MS and SAXS

Kazumi Saikusa[1], Naoyuki Kuwabara[2], Yuichi Kokabu[1], Yu Inoue[1], Mamoru Sato[1], Hiroshi Iwasaki[3], Toshiyuki Shimizu[2], Mitsunori Ikeguchi[1], Satoko Akashi[1]

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Keywords:
small-angle X-ray scattering, dummy residue model, intrinsically disordered protein, ion mobility mass spectrometry

Novel aspects:
First application to evaluate the experimental CCS values of IDPs using SAXS dummy residue models.

Abstract:
It has now been recognized that intrinsically disordered proteins (IDPs) play important roles as hubs in intracellular networks, and their structural characterization is of significance. Due to their highly dynamic features, it is not easy to investigate their structures only by conventional methods, such as X-ray crystallography. In the present study, we tried to characterize IDP complexes using ion mobility mass spectrometry (IM-MS) in combination with small-angle X-ray scattering (SAXS). This method enables structural characterization even of proteins that have difficulties with crystallization. With this method, we have characterized the *Schizosaccharomyces pombe* Swi5-Sfr1 complex, which is expected to have a long disordered region at the N-terminal portion of Sfr1. Here we contrasted the structural characteristics of the intrinsically disordered (ID) region in the gas phase with those in the solution phase proved by SAXS.
The collision cross sections (CCSs) and low-resolution structures were analyzed for the Swi5-Sfr1 and Swi5-Sfr1C complexes by traveling-wave IM-MS and SAXS. Sfr1C is a mutant of Sfr1 lacking the ID region of the N-terminal 177 amino acid residues, and the atomic-level structure of the Swi5-Sfr1C complex has already been determined by X-ray crystallography.

NanoESI-MS of Swi5-Sfr1 and Swi5-Sfr1C revealed that Swi5 (9.7 kDa) formed 1 : 1 complexes with Sfr1 (33.5 kDa) and Sfr1C (14.1 kDa), respectively. The SAXS experiments suggested that these complexes exhibited the elongated shapes, especially for the Swi5-Sfr1 complex. The CCS values of multiply-charged ions of the complexes were obtained by converting their arrival times in the ion mobility region. The CCS distribution of the Swi5-Sfr1C complex indicated that there were four conformers exist with the centroid CCSs of 2100, 2310, 2870 and 3720 A^2. As for the Swi5-Sfr1 complex, three conformers with the centroid CCSs of 3160, 6380, and 7940 A^2 were found. The most dominant ions observed in the ESI mass spectra of the Swi5-Sfr1 and Swi5-Sfr1C complexes were with the most compact shapes.
To evaluate the experimental CCS values, low-resolution dummy residue models for the Swi5-Sfr1C and Swi5-Sfr1 complexes were constructed with the SAXS data and their theoretical CCS values were calculated. Upon CCS calculation, a dummy residue radius of 5.8 A was adopted, which was obtained by comparing the X-ray and dummy-residue model structures constructed based on the PDB atomic coordinates of 10 model proteins.
The experimental CCS value of the most dominant ion observed in the ESI mass spectrum of the Swi5-Sfr1 complex was extremely smaller than the theoretical value. In the case of Swi5-Sfr1C, the compaction level was not as high as Swi5-Sfr1. This implies that a considerable compaction occurred for the ID region of the complex during IM-MS measurement. Furthermore, this study indicates that the experimental CCS values of proteins containing long ID regions can be evaluated with the theoretical CCS values calculated for their SAXS low-resolution solution structures.

Oral Session

Thursday, 20th September

Main Hall

15:00 – 17:00

Session 36: Advances in Ion Mobility Mass Spectrometry

Chair: Joseph A Loo (University of California-Los Angeles, USA)

S36-1600

16:00 – 16:20

Probing Protein Quaternary Structures by Surface Induced Dissociation and Ion Mobility - Mass Spectrometry

Mowei Zhou[1,2], Shai Dagan[1], Vicki H Wysocki[1,2]

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Keywords:
surface induced dissociation, ion mobility, protein complexes

Novel aspects:
First comprehensive presentation illustrating the advantages of combining surface induced dissociation with ion mobility to study large non-covalent protein complexes

Abstract:
Quaternary structures of protein complexes are highly important to their biological functions. The combination of ion mobility (IM) and mass spectrometry (MS) , in which both conformational and molecular weight information of the assemblies can be obtained, greatly expanded the application of MS in structural biology. Subunit organization of protein complexes can be studied with tandem mass spectrometry by activating the original assemblies and releasing sub-structures in a controlled manner. The major challenge of applying this method is to overcome the undesired unfolding of protein subunits in the commonly used gas collision activation (collision induced dissociation, CID) which usually results in the loss of information for the native conformation.

We present here the incorporation of a custom surface induced dissociation (SID) device in a modified quadrupole/IM/time-of-flight mass spectrometer. The SID device is positioned either before IM cell (SID-IM) which provides conformational information on product ions, or after IM cell (IM-SID) for shape selected activation. With collisional cross sections (CCSs) of remaining precursor ions after activation measured using the SID-IM setup, C-reactive protein (CRP) pentamer is shown to undergo significant structural rearrangement and unfolding upon CID. Major monomer product ions in CID are partially unfolded. Instead, CRP pentamer dissociates rapidly into compact monomers in SID without remarkable change in collisional cross section of the precursors. This illustrates the advantage of using SID to activate protein complexes with minimum disruption in their conformation so that the product ions are informative to their native architecture. Additionally, SID-IM simplifies interpretation of tandem MS spectra because of the separation of oligomeric subunit products which could overlap in mass-to-charge, elucidated by SID spectra of several protein complexes. With the IM-SID setup, SID of selected conformations of a protein complex can be performed. As an example, two conformational ensembles of serum amyloid P (SAP) decamers are separated and activated by SID, which revealed differences in subunit packing between the two structures.

Oral Session

Thursday, 20th September

Main Hall

15:00 – 17:00

Session 36: Advances in Ion Mobility Mass Spectrometry

Chair: Joseph A Loo (University of California-Los Angeles, USA)

S36-1620

16:20 – 16:40

Energy-resolved ion mobility tandem mass spectrometry: A new approach for probing fragmentation pathways involving ion isomerization

Yayoi Hongo[1], Takemichi Nakamura[1], Takae Takeuchi[2], Shunya Takahashi[1], Hiroyuki Koshino[1]

[1]RIKEN, Wako, Japan, [2]Nara Women's University

Keywords:

energy-resolved ion-mobility tandem mass spectrometry (ER-IMS/MS), collision cross section, small molecular characterization

Novel aspects:

Energy-resolved ion mobility tandem mass spectrometry was applied to a macrocyclic system and found to be a useful tool for probing the fragmentation involving acyclic intermediate.

Abstract:

In tandem mass spectrometry (MS/MS), activated gas-phase ions undergo a variety of reactions that can be categorized as fragmentation and isomerization. Among various reaction pathways from activated even-electron ions, isomerization may be no less common than fragmentation. Structure specific isomerization (or rearrangement) should give us rich information on ionic structure. However, ion isomerization in MS/MS has not been well documented or studied in the past, partly because rather few direct methods for probing such reactions have readily been available.

While on the other hand, ion mobility spectrometry (IMS) has widely been recognized as a useful tool for studying gas-phase ion structure. Consequently, recent advancements in hybridization of IMS and MS/MS devices should give us unique opportunity for the study of isomerization upon activation of gas-phase ions. Here we report a new approach to probe fragmentation pathways involving ion isomerization using ion mobility analysis combined with energy-resolved tandem mass spectrometry (ER-IMS/MS).

Ring-opening reactions of cyclic molecules are considered to be one of the distinct examples of ion isomerization. To demonstrate the proof of concept of ER-IMS/MS, we've investigated fragmentation behavior of a compound, cyclo-di-SIP-B ($C_{50}H_{56}O_{10}$), a derivative of a natural-product Phomopsin B[1] and related compounds. The structure of cyclo-di-SIP-B includes 20-membered-ring corresponding to dimer of 'SIP-B' unit. The ER-IMS/MS experiments were conducted by using a Synapt G2 HDMS, IMS Q-TOF instrument (Waters), equipped with an ESI source.

The sodiated molecule of cyclo-di-SIP-B ($[M+Na]^+$, *m/z* 839) gave a major fragment ion at *m/z* 431 ($[C_{25}H_{28}O_5\,Na]^+$) corresponding to the mass of 'SIP-B' unit, generation of which requires cleavages at two sites in the ring structure, i.e. ring-opening to give an acyclic structure and 2nd cleavage to give the fragment ion. In the ER-IMS/MS experiment, in which precursor ion was collisionally activated prior to entering to the mobility cell, a single peak was detected in the mobiliogram of *m/z* 839 with lower collision energies (CE). On the other hand, interestingly, a second peak of *m/z* 839 appeared at a little larger drift time at higher CE. The second peak is attributable to an isomerized precursor as it showed substantially larger collision cross section (CCS) compared to that of the original cyclic ion. In other words, the ion with larger CCS may be attributable to the hypothetical ring-opened 'intermediate' of the fragmentation pathway from *m/z* 839 to 431. In order to confirm the hypothesis, we've designed a double CID ER-IMS/MS experiment. The mass analyzed *m/z* 839 precursor were subjected to the first CID to before entering to the mobility cell. After the mobility separation, the isomers of *m/z* 839 were fragmented in the 2nd CID cell. The energy-resolved MS/MS spectra of two isomers were virtually identical showing dominant fragment ion at *m/z* 431; the larger CCS isomer was suggested to be an 'intermediate' of fragmentation from *m/z* 839 to 431. There appeared to be a few eV difference in CID onset for the original cyclic structure and the acyclic isomer. The energy difference may be associated with the energy requirement for the ring-opening reaction, or isomerization prior to fragmentation.

Combined with theoretical assessment of CCS and energetics of reactions, ER-IMS/MS can be a powerful means to probe the ion fragmentation pathway which contains ion isomerization in mass analyzer. Potential application of ER-IMS/MS is further explored.

[1] Izuchi, Y., Koshino, H., Hongo, Y., Kanomata, N., Takahashi. S., 2011, *Org. Lett.*, 13, 3360-3363

S36-1640

16:40 – 17:00

Understanding protein-DNA Interactions and Tumorigenic Mutations in IDPs: IM-MS rises to the challenge

Ewa Jurneczko[1], Faye Cruickshank[1], Penka Nikolova[2], Ian Campuzano[3], Michael Morris[3], Perdita E Barran[1]

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Keywords:
Ion Mobility-Mass Spectrometry, p53-DNA Interactions

Novel aspects:
First reported use of (ion mobility) mass spectrometry to systematically examine protein-DNA interactions and Tumorigenic Mutations in IDPs

Abstract:

Introduction

Common approach to understanding protein function is to 'solve' its structure and subsequently probe interactions between the protein and its binding partners. The first part of this approach is non-trivial for proteins where localised regions or even their entire structure fail to fold into a 3-D structure and yet they possess function. These so called intrinsically or inherently disordered proteins (IDPs) or intrinsically disordered regions (IDRs) constitute up to 40 % of all expressed proteins, and a much higher percentage in proteins involved in the proliferation of Cancer. The tumour suppressor p53 has an intimate and multifaceted role in the cell cycle and that is reflected in its equally complex structure. The full length protein has a modular domain structure, consisting of DNA-binding and tetramerisation domains, flanked by intrinsically disordered regions at both the amino and caroboxy termini, which poses a challenge for the structural biologists. Here we use ion mobility mass spectrometry to assess the relative disorder of p53, in a complex with different lengths of DNA and also mutants of the DNA binding domain.

Methods

Wild type p53 and mutants of the core DNA-binding domain (amino acid residues 94-312) were dialysed in 50 mM ammonium acetate for 2 h at 4 °C. 10% by volume isopropanol was added prior toanalysis by mass spectrometry to aid desolvation. Non-native samples were sprayed from buffered solutions containing 10% by volume isopropanol and 1 % formic acid. DNA oligomers contained the following sequences : 12mer : GGAACATGTTCC and 20mer : GAACATGTTCGAACATGTTC which are derived from the p53 consensus binding site. All oligomers were annealed by heating them to 95° C for 5 min. Data were obtained on a TW IM-MS (SynaptHDMS, Waters, Manchester, UK) in positive ionisation mode and a home built linear DT IM-MS instrument. In the linear DT IM-MS instrument data was also obtained at different buffer gas temperatures to examine the thermal unfolding of these proteins.

Preliminary Data

Mass spectrometry studies of WT p53 and mutants of the DNA binding domain following nano-electrospray from buffered conditions show a wide charge state distribution (8 *z* 18) formonomeric species of the general form $[M+zH]^{z+}$, which we highlight as signature behaviour of IDPs *in vacuo* as reported by mass spectrometry. The collision cross-sections of the monomer are observed to increase with increasing charge in a stochastic fashion attributed to protein unfolding due principally to Coulombic repulsion, which can be attributed to the 'plasticity' of these IDPs. Differences in unfolding transitions between the WT and mutated samples can be characterized. The H115N mutation located at the boundary of the IDR loop I appears to be less structured, favoring the more extended conformations when compared with WT p53. The structural 'hot spot' mutation in the loop III region R249A shows less population in unfoldomers and the structure appears even tighter, contrary to what expected.

The majority of the mutations occur in the core domain which contains the sequence-specific DNA binding activity of the p53 protein. And they result in a loss of DNA binding. The DNA binding is critical for the biological activity of p53, and provides a frame work for understanding how mutations inactivate it. In a complex with DNA, p53 core domain binds all available consensus binding sites in both DNA constructs. WTp53 binds to the 12merDNA as a dimer and as four monomers to the 20mer DNA. The interaction is highly cooperative, and relies on the recognition of DNA sequences by the core domain and on the tetramerization of the protein, which is heavily mediated by the presence of DNA. These results are interpreted in terms of the biological activity and also in terms of a view of the allosteric regultion in these proteins.

Oral Session

Thursday, 20th September

Room A

15:00 – 17:00

Session 37: Challenges in High Resolution and High Accuracy Mass Measurement Mass Spectrometry

Chair: Evgeny N Nikolaev (The Institute for energy problems of chemical physics Russian Academy of Sciences, Russia)

S37-1500

15:00 – 15:40

[Keynote Lecture] Mass Resolution and Mass Accuracy: How Much is Enough?

Alan G Marshall, Greg T Blakney, Nathan K Kaiser, Yuan Mao, Amy M McKenna, Ryan P Rodgers, Brian M Ruddy, Christopher L Hendrickson

Florida State University,Tallahassee, Florida, U.S.A.

Keywords:
Fourier Transform Mass Spectrometry, FTMS

Novel aspects:
Hardware and software developments leading to higher mass resolving power, mass resolution, and mass accuracy. Minimum resolving power for unique assignment of elemental composition and amino acid composition.

Abstract:
Accurate mass measurement must be preceded by the highest possible mass resolution, to ensure that only a single elemental composition contributes to the mass spectral peak in question. Although mass resolution is conventionally defined as the closest distinguishable separation between two peaks of equal height and width, the required mass resolving power is ~10x higher for equal width peaks whose peak height ratio is 100 : 1. Ergo, minimum resolving power requires specification of maximum dynamic range, and is thus 10-100x higher than the conventional definition.

The highest available broadband mass resolving power and mass accuracy is from Fourier transform ion cyclotron resonance mass spectrometry. I shall describe how, over the past five years, FT-ICR MS mass accuracy has improved by about an order of magnitude, based on higher magnetic field strength, conditional co-adding of time-domain transients, better mass calibration (spectral segmentation ; inclusion of a space charge term ; radially dispersed excitation ; phase correction to yield absorption-mode display ; and new azimuthal and axial ICR cell segmentation designs. Work supported by the National Science Foundation (DMR-06-54118 ; CHE-1016942 ; CHE-1019193 and the State of Florida.

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Valeja, S. G. ; Kaiser, N. K. ; Xian, F. ; Hendrickson, C. L. ; Rouse, J. C. ; Marshall, A. G. "Unit Mass Resolution for an Intact 148 kDa Therapeutic Monoclonal Antibody by FT-ICR Mass Spectrometry, "*Anal. Chem.***2011**, *83*, 8391-8395.

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Vladimirov, G. ; Hendrickson, C. L. ; Blakney, G. T. ; Marshall, A. G. ; Heeren, R. M. A. ; Nikolaev, E. N. "Fourier Transform Ion Cyclotron Resonance Mass Resolution and Dynamic Range Limits Calculated by Computer Modeling of Ion Cloud Motion, *J. Am. Soc. Mass Spectrom.***2012**, *23*, 375-384.

Oral Session

Thursday, 20th September

Room A

15:00 – 17:00

Session 37: Challenges in High Resolution and High Accuracy Mass Measurement Mass Spectrometry

Chair: Evgeny N Nikolaev (The Institute for energy problems of chemical physics Russian Academy of Sciences, Russia)

S37-1540

15:40 – 16:00

Enhanced Fourier transform and filter diagonalization method mass spectrometry for top-down analysis of antibodies and petroleomics

Yury O Tsybin, Ünige A Laskay, Luca Fornelli, Konstantin O Zhurov, Anton N Kozhinov

Ecole Polytechnique Federale de Lausanne, Switzerland

Keywords:
top-down mass spectrometry ; Fourier transform mass spectrometry ; Orbitrap ; filter diagonalization method ; petroleomics

Novel aspects:
Enhanced Fourier transform and filter diagonalization method-based signal processing accelerate high resolution and high mass accuracy data acquisition in FT mass spectrometry improving petroleomics and top-down structural characterization of antibodies

Abstract:
Fourier transform mass spectrometry (FTMS) provides superior resolution and mass accuracy among the palette of the MS instruments. However, comprehensive structure analysis of molecules and macromolecules present in extremely complex biological and environmental samples and/or performed under time-constrained experimental conditions demands a substantial increase in the acquisition speed of high-resolution and high mass accuracy MS data. For this reason, absorption mode spectra representation has been implemented in Orbitrap FTMS as a part of enhanced FT (eFT) signal processing and its development is in progress in FT-ICR MS. On the other hand, filter diagonalization method (FDM) is capable of overcoming the FT resolution limitation. Recently, we described the first implementation of FDM for FT-ICR MS. [1,2] Here, we demonstrate performance optimization of hybrid Orbitrap Elite FTMS in the top-down analysis of intact proteins as well as feasibility and limitations of Orbitrap FTMS compared to FT-ICR MS in routine analysis of petroleum-type samples. In addition, we examine performances of FT, eFT, and FDM approaches for the transient data treatment.

Proteins were obtained from commercial sources or expressed in-house. Complex molecular mixtures, including crude oil fractions, were obtained through collaboration. MS and MS/MS experiments were performed using 10 T LTQ ICR and LTQ Orbitrap Elite FT mass spectrometers (both Thermo Scientific) . Ionization was achieved by atmospheric pressure electrospray or photoionization, either in direct infusion or in on-line liquid chromatography (LC) -MS/MS approach. The instrumental parameters and operation were controlled by standard and advanced user interface data acquisition software allowing acquisition of transient signals of user-defined length. Data treatment using FT and FDM signal processing methods was performed using in-house developed Python-based software running on a multi-core workstation.

First, we examined performance gain of eFT and FDM signal processing for the data treatment compared to the conventional magnitude mode FT. The results indicate that FDM-based MS presents a number of advantages compared to other signal processing methods, including FT and eFT. [1] In contrast to FT-ICR MS performed at 10 T, FDM-based signal processing of the same data requires transients containing only 1 isotopic beat for multiply charged 10-20 kDa proteins to provide their isotopic distribution resolution. Importantly, FDM-based MS confirms its applicability in the case of high spectral complexity. [1] Briefly, in case of extremely complex mixtures the application of FDM for the analysis of time-domain transient signals in FT-ICR MS reduces the required transient length by a factor of 2-4. Furthermore, FDM MS provides complementary molecular structure information, such as peptide fine isotopic structures, on a LC time scale. [2]

We will demonstrate further method and technique development of FDM MS methodology for FT-ICR MS and extent this methodology to the Orbitrap FTMS. Particularly, we will present the application of eFT and FDM MS to the analysis of intact proteins, including monoclonal antibodies, by electron transfer dissociation (ETD) -based top-down approach. [3] In the area of petroleomics, the new hybrid high-resolution Orbitrap FTMS platforms with eFT bridge the resolution gap with FT-ICR MS in a wide *m/z* range. We will apply FDM MS to push the upper mass limit for separation of closely spaced molecular peaks in Orbitrap FTMS-based petroleomics. Particular attention will be paid to the mass accuracy challenge in Orbitrap FTMS-based petroleomics with FT, eFT, and FDM signal processing. We will also discuss the current limitations of the FDM-based MS and some technical details of its current implementation.

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3. Tsybin Y. O., et al. *Anal. Chem.* (2011) , 83 (23) , 8919-8927

Oral Session

Thursday, 20th September

Room A

15:00 – 17:00

Session 37: Challenges in High Resolution and High Accuracy Mass Measurement Mass Spectrometry

Chair: Evgeny N Nikolaev (The Institute for energy problems of chemical physics Russian Academy of Sciences, Russia)

S37-1600

16:00 – 16:20

Characterization and Selective Classification of Petroleum Crude Oils using High Resolution Time-of-Flight Mass Spectrometry

Clecio Klitzke[2], Marcos Eberlin[2], Kevin Siek[1], Jeffrey S Patrick[1], Joe Binkley[1], Yuri Corillo[2]

[1]LECO Corporation, [2]UNICAMP, ThoMSon Mass Spectrometry Laboratory, Campinas, São Paulo Brasil

Keywords:
Resolution, Petroleomics, Time-of-flight, FTMS, Heteroatoms

Novel aspects:
This work demonstrates the practical retention of key information from petroleum characterization in translation form FTMS to HR TOF including heteroatom contributions and the definition of key petroleum attributes.

Abstract:
Crude oil samples from different sources and having different quality chemical attributes are examined by high performance time of flight mass spectrometry. The data are evaluated using an software specifically designed to process the TOF data an access molecular level information of the oil samples through the high resolution data. Crude oil samples are ionized using electrospray ionization by direct infusion and the mass analyzes is performed at a variety of resolving powers on FT-ICR MS (7.2 T) and on a high resolution time of flight (HRT) mass spectrometer. Specifically, at 50,000 and ca. 90,000 on the HRT and 100,000, 200,000 and 400,000 on the FT-ICR MS systems. These data are used to classify the crude oil samples based on their most abundant content of heteroatomic species as well other measurements of significance to petroleum processing. Comparison is made between samples, resolving power conditions and with real and theoretical data obtained from other measurements. The ability to selectivity access the significant descriptors of crude oil pertinent to source identification and processing quality is demonstrated. The data were acquired on a time of flight mass spectrometer using acquisition times of less than 10 minutes per sample with a simple sample preparation. The information content of significance to petroleum researchers and engineers that is retained at the various resolving powers is compared and contrasted with the information content being retained on the HRT compared with 400,000 resolving power on the FTMS.

Oral Session

Thursday, 20th September

Room A

15:00 – 17:00

Session 37: Challenges in High Resolution and High Accuracy Mass Measurement Mass Spectrometry

Chair: Evgeny N Nikolaev (The Institute for energy problems of chemical physics Russian Academy of Sciences, Russia)

S37-1620

16:20 – 16:40

Detailed structure analysis of fluorinated polymer by means of pyrolysis comprehensive two-dimensional gas chromatography / high-resolution time-of-flight mass spectrometry

Yoji Nakajima, Yuko Arinami, Tsuguhide Isemura, Kiyoshi Yamamoto
Asahi Glass Co., Kanagawa, Japan

Keywords:
TOFMS GCxGC polymer high-resolution

Novel aspects:
The usefulness of Py-GCxGC/HRTOFMS for chemical structure analysis of fluorinated polymers was demonstrated. Detailed structure of the polymer such as sequence and end-group were successfully identified by using accurate mass.

Abstract:
We demonstrate the usefulness of pyrolysis (Py) comprehensive two-dimensional gas chromatography (GCxGC) / high-resolution time-of-flight mass spectrometry (HRTOFMS) for chemical structure analysis of fluorinated polymers. New fluorinated polymers are rapidly emerging in recent years to meet requirements of advanced applications in many fields. Introduction of fluorine into polymers leads to drastic improvements in several properties, such as chemical stability, electric and optical properties. However, there are peculiar technical difficulties for chromatography and mass spectrometry of fluorinated polymers. For example, in relation to chromatography, fluorinated polymers are often insoluble in common organic solvent. In this case, Py-GC/MS is generally useful for structure analysis of insoluble polymers. However, pyrolysis of fluorinated polymers often generates great numbers of unidentified peaks which cannot be separated by one-dimensional GC.

Recently, comprehensive GCxGC has been developed and applied to separate complex samples mainly in the fields of geochemistry and environmental chemistry. Separation ability of GCxGC is several tens times higher than one-dimensional GC. In relation to mass spectrometry, most of pyrolysis products of fluorinated polymers are unidentified and not included in commercial database of mass spectra. In this regard, accurate mass spectrometry by using HRTOFMS appears as a useful technique in structural analysis of such unidentified compounds. In this study, combination of GCxGC and HRTOFMS were used to resolve the problems of separation and identification of the pyrolysis products. Our Py-GCxGC/HRTOFMS system consists of a microfurnace pyrolyzer (Frontier Lab PY-2010D) and a gas chromatograph (Agilent GC7890) equipped with a high-resolution time-of-flight mass spectrometer (Jeol AccuTOF) and a GC×GC jet modulator (Zoex KT2006) . Typical measurement conditions of the system are as follows. Pyrolysis temperature is 600 °C. GC columns are DB-5 for the first dimension and DB-17 for the second dimension. GC×GC modulation is performed with liquid nitrogen cooling during 6 sec. GC oven temperature is programmed as 40 °C (5 min) , ramp to 300 °C at 5 °C /min. Helium is used as a carrier gas at 1.5 ml/min. HRTOFMS is operated at the acquisition rate of 25 Hz. Mass spectra are collected from *m/z* 35 to 800.

We have applied the Py-GCxGC/HRTOFMS system to structural analysis of various fluorinated polymers. The advantages of application to structural analysis of fluoroacrylate (FA) /methylmethacrylate (MMA) copolymer are clearly described. GC×GC chromatogram of FA/MMA copolymer shows several hundred well separated peaks. GC×GC chromatogram pattern had good reproducibility. Many of those peaks couldn't have been separated and detected by means of one-dimensional GC. In addition to the separation ability, sensitivity of GC×GC is also higher than that of one-dimensional GC because the GC×GC modulation process makes the peaks sharper. The first dimension of GC×GC represents the separation according to boiling points and the second dimension represents polarity. FA derived compounds were detected in the lower polarity region of the GC×GC chromatogram, and fully separated from MMA derived compounds detected in the higher polarity region. The advantages of GC×GC include enhanced identification due to well ordered and classified detection of the compounds in GC×GC chromatogram. Accurate mass spectra of the compounds obtained by HRTOFMS can provide us not only molecular weight and fragmentation information but also elemental composition information. By combining those peaces of information together, many compounds including detailed structure information of the polymer such as sequence and end-group were successfully identified. In addition, the Py-GCxGC/HRTOFMS seems to be a powerful tool for detailed structure analysis of not only fluorinated polymers but also some other advanced synthetic polymers.

Oral Session

Thursday, 20th September

Room A

15:00 – 17:00

Session 37: Challenges in High Resolution and High Accuracy Mass Measurement Mass Spectrometry

Chair: Evgeny N Nikolaev (The Institute for energy problems of chemical physics Russian Academy of Sciences, Russia)

S37-1640

16:40 – 17:00

Dynamically harmonized FTICR cell with specially shaped electrodes for compensation of inhomogeneity of the magnetic field.

Yury Kostyukevich[1], Gleb Vladimirov[1], Eugene Nikolaev[1,2,3]

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Keywords:
FTICR, dynamically harmonized cell, simulation

Novel aspects:
Methods of compensation of the magnetic field inhomogeneity inside a dynamically harmonized FTICR cell by introducing specific electric field corrections

Abstract:
The recently introduced FTICR ion trap with dynamic harmonization shows the highest resolving power ever achieved both for ions with moderate masses 500-1000 Da (peptides) as well as ions with very high masses of up to 200 kDa (proteins). Time of transient duration corresponding to the time of synchronous motion of the ion clouds with m/z around 500 excited to cyclotron motion is more than 5 minutes and seems to be limited only by the vacuum inside FT ICR cell. Such results were obtained for superconducting magnets of very high homogeneity of the magnetic field.

In superconducting magnets used in FTICR mass spectrometry the inhomogeneity of the magnetic field in its axial direction prevails over the inhomogeneity in other directions and should be considered as the main factor influencing the synchronous motion of the ion cloud [1]. The inhomogeneity leads to a dependence of the cyclotron frequency on the amplitude of axial oscillation in the potential well of the ion trap. As a consequence the ions in an ion cloud become dephased, which leads to a decrease in the resolving power.

Ion cyclotron frequency is also affected by the radial component of the electric field. Hence by appropriately adjusting the electric field one can compensate the inhomogeneity of the magnetic field and align the cyclotron frequency in the whole range of amplitudes of z-oscillations. Such compensation has recently been experimentally demonstrated for Gabrielse's type FT ICR trap [2]. Here a method of magnetic field inhomogeneity compensation in a dynamically harmonized FT ICR cell is presented, which is based on the introduction of additional electrodes into the cell shaped in such a way that the averaged electric field created by these electrodes produces a counterforce to the forces caused by the inhomogeneous magnetic field.

Realistic computer simulation clearly demonstrates the possibility of the proposed cell design to sufficiently (by the factors of 5-10) increase a time of synchronic ion motion for the magnetic field of the following dependence on z coordinate: B=B0 (1+a*z*z). Such inhomogeneity has the main influence on cyclotron frequency for magnets used in FTICR mass spectrometry.

The authors acknowledge the support from the Russian Foundation of Basic Research (grant 10-04-13306), from the Russian Federal Program (state contracts 14.740.11.0755, 16.740.11.0369), and from the Fundamental Sciences for Medicine Program of the Russian Academy of Sciences.

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2) Nathan K. Kaiser, Joshua J. Savory, Amy M. McKenna, John P. Quinn, Christopher L. Hendrickson, and Alan G. Marshall Electrically Compensated Fourier Transform Ion Cyclotron Resonance Cell for Complex Mixture Mass Analysis Anal. Chem., 2011, 83 (17), pp 69076910

Oral Session

Thursday, 20th September

Room B-1

15:00 – 17:00

Session 38: Mass Spectrometry for Metabolic Diseases

Chair: Makoto Yoshino (Kurume University, Japan)
Seiji Yamaguchi (Shimane University, Japan)

S38-1500

15:00 – 15:20

LC/MS/MS analysis of protein-bound uremic toxins in hemodialysis patients

Toshimitsu Niwa[1], Yoshiharu Itoh[2], Atsuko Ezawa[2], Kaori Kikuchi[2], Yoshinari Tsuruta[3]

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Keywords:
uremic toxin, LC/MS/MS, hemodialysis

Novel aspects:
This study describes simultaneous LC/ESI-MS/MS measurement of 12 uremic solutes in hemodialysis patients, and first demonstrates the protein-binding of indoxylglucuronide, p-cresylglucuronide, phenyl sulfate, phenylglucuronide, and 4-ethylphenyl sulfate in uremic serum.

Abstract:
Introduction: Cardiovascular disease (CVD) isprevalent in patients with chronic kidney disease (CKD). In hemodialysis (HD) patients, some protein-bound uremic toxins are considered to be associated with CVD. However, it is not yet known which uremic toxins are important in terms of endothelial toxicity. **Methods:** Serum samples were obtained from 45 hemodialysis (HD) patients before and after HD. Total and free serum concentration of indoxyl sulfate, indoxyl glucuronide, indoleacetic acid, p-cresyl sulfate, p-cresyl glucuronide, phenyl sulfate, phenyl glucuronide, phenylacetic acid, phenylacetyl glutamine, hippuric acid, 4-ethylphenyl sulfate, and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) were simultaneously measured by liquid chromatography/electrospray ionization-mass spectrometry/mass spectrometry (LC/ESI-MS/MS). The effects of these solutes at their pre-HD mean and maximum serum concentrations on reactive oxygen species (ROS) production in human umbilical vein endothelial cells (HUVEC) were measured with a ROS probe. **Results:** Serum levels of 11 solutes except 4-ethylphenyl sulfate were significantly increased in HD patients compared to healthy subjects. All the 12 solutes showed more or less protein-binding. Especially, indoxyl sulfate, p-cresyl sulfate, CMPF, and 4-ethylphenyl sulfate showed high protein-binding ratios (more than 95%) and low reduction rates by HD (less than 35%). Indoxyl sulfate at its mean and maximum pre-HD serum concentration even with 4% albumin stimulated ROS production in HUVEC most intensely, followed by CMPF. **Conclusion:** Serum levels of 11 protein-bound uremic toxins were increased in HD patients. Indoxyl sulfate, p-cresyl sulfate, and CMPF could not be removed efficiently by HD due to its high protein-binding ratio. Indoxyl sulfate most intensely induced endothelial ROS production, followed by CMPF.

S38-1520
15:20 – 15:40

Early indicator of hepatic fibrosis in patients with chronic hepatitis C: discovery, assay optimization and clinical validation

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Keywords:
hepatic fibrosis, fibrinogen alpha chain,

Novel aspects:
A novel biomarker candidate to detect hepatic fibrosis related to hepatis C virus at an early stage was discovered by MS, and simple and high-throughput assay was developed.

Abstract:
When the damage to the liver is chronic, excess fibrous connective tissue accumulates irrespective of the etiology. With advancements in the treatment of chronic liver diseases, the accurate assessment of hepatic fibrosis is a pivotal need for successful individual management of patients. Liver biopsy has long been the gold standard for assessing the degree of liver fibrosis. However, it is invasive and also prone to sampling errors and/or interobserver variation. Therefore, there is a need for non-invasive biomarkers for hepatic fibrosis. Proteome analyses should be a promising tool to search for novel fibrosis markers.
Although current proteomic technologies permit identification of new biomarkers, diagnostic test development remains challenging. Indeed, a large number of marker candidates detected and identified by MS-based proteomic methods are left untested because an appropriate ELISA for a validation study is not available.
We previously identified a 5.9 kDa peptide fragment of fibrinogen α C-chain (FIC 5.9) as a novel biomarker candidate for heavy drinking. Since our initial identification of FIC5.9, the same peptide has been reported to be of diagnostic value in a variety of pathological conditions including cancers and inflammatory diseases. To establish diagnostic values of FIC 5.9 in a large scale, it is mandatory to develop simple and reproducible assay system.
Since FIC5.9 was first detected and identified by MALDI-TOF, a stable isotope-labeled internal standard dilution (SID-MS) assay was the initial option for quantitative determination of FIC5.9 concentrations in serum samples. We constructed a stable isotope-labeled internal standard dilution (SID-MS) assay to determine serum FIC 5.9 levels. Although the SID-MS FIC 5.9 determinations were reproducible, other methods were desirable in terms of throughput and feasibility.
Since the size of FIC 5.9 was outside the detection limit of MS combined with MRM or SRM, we decided to construct immunoassays for FIC 5.9. FIC 5.9 is a fragment of the fibrinogen alpha C chain, and it is likely that serum samples contain a number of fibrinogen fragments of different sizes. It was not an easy task to obtain an antibody that can specifically react with one particular fragment. For this purpose, two monoclonal antibodies specific to the N and C-termini of the 5.9-kDa peptide were used to develop a FIC5.9 sandwich ELISA. The assay was evaluated by comparing the results with those obtained by the stable isotope-labeled dilution mass spectrometry (SID-MS).
The ELISA results correlated with the SID-MS findings (slope=0.795, intercept=-0.011, r (2) =0.908) and the performance of the ELISA was satisfactory in terms of recovery (98.5-103.0%) and within-run (1.4-4.7%) and between-day (2.8-8.4%) reproducibility.
We then applied this immmunoassays to serum samples obtained from patients with chronic liver diseases of various etiologies. A total of 371 serum samples obtained from 65 patients with chronic HBV infection, 126 patients with chronic HCV infection and 180 healthy volunteers were analyzed by the ELISA assay.
Serum levels of FIC 5.9 in healthy subjects were 12.1 $\pm$ 1.7 mg/mL, whereas those for patients with chronic hepatitis C were 5.1 $\pm$ 2.6 mg/mL ($p<0.001$). Interestingly, FIC 5.9 levels were markedly decreased in as early as F 1 (namely very early) stage of chronic hepatitis C (5.5 $\pm$ 1.7 mg/mL ; $p<0.001$), whereas other representative conventional markers to assess hepatic fibrosis (type IV collagen and hyaluronic acid were not.
Measurement of serum FIC 5.9 levels could be a sensitive indicator to detect hepatic fibrosis at an early stage in chronic hepatitis C. A larger-scale multicenter study is currently underway to further confirm the results obtained in this preliminary study.

S38-1540

15:40 – 16:00

Liquid chromatography-mass spectrometry based analysis of the cerebrospinal fluid metabolome for the study of inborn errors of metabolism

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Keywords:
Metabolomics, cerebrospinal fluid, liquid chromatography coupled to mass spectrometry, inborn errors of metabolism.

Novel aspects:
LC/MS based metabolomics of CSF provides new insights into the pathogenic mechanisms of known inborn errors of metabolism, and is also relevant for the stratification of patients with unexplained encephalopathies.

Abstract:

Introduction

Many complex neurological disorders remain of unknown etiology despite extensive biochemical and genetic work-up. These "unexplained encephalopathies" may be caused by inborn errors of metabolism (IEM). Diagnosis of IEMs can usually be accomplished by the biochemical analysis of biofluids (blood and urine in most cases) to highlight the consequences of an enzymatic or a transport protein defect. However, biochemical analyses available at clinical chemistry laboratories are restricted to well-known metabolites, and many metabolic pathways remain unexplored. By enabling the concomitant detection of a wide range of metabolites in biological fluids in a single analysis, metabolomics appears to be a relevant tool for the diagnosis of IEMs [1-3]. Regarding disorders with predominant nervous system dysfunction, the cerebrospinal fluid (CSF) is the body fluid more likely to be altered because of its proximity with neuronal and glial cells. In the present pilot study, we aimed at evaluating liquid chromatography coupled to mass spectrometry (LC/MS) -based CSF metabolomics for the study and characterization of IEMs involving the nervous system.

Material and methods

Patients. For this study, 79 CSF samples were selected from a biobank collected from adult patients seen in the neurometabolic unit of the Pitie-Salpetriere Hospital. CSF sample collection was realized after written informed consent, in accordance with french ethical rules. Selected CSF samples were classified into 4 categories: (i) 7 Negative controls without progressive neurological disease, (ii) 12 patients with well-defined neurometabolic disorders, (iii) 60 patients having unexplained encephalopathies, and (iv) 15 patients with various neurodegenerative diseases.

LC/MS based metabolomics. Hundred µl of CSF samples were deproteinized in microcentrifuge tubes by adding 300 µl of methanol. Samples were vortexed and centrifugated. Supernatants were evaporated to dryness under nitrogen and resuspended in 100 µl of water containing 0.1% of formic acid. Five µl of a solution of internal standards were added to all samples. In addition, a quality control CSF sample was injected every 10 samples in order to evaluate the analytical error at the level of each metabolite. Samples were analyzed by ultra performance liquid chromatography coupled to an ESI-LTQ-Orbitrap operated in positive and negative modes. Data processing and treatment were performed as previously described [4].

Results

The developed LC/MS and processing methods led to the identification of 104 metabolites in the CSF. CSF analysis revealed a specific abnormal fingerprint in 4 out of 12 patients with confirmed metabolic diseases, emphasizing that the possibility to measure metabolites not previously analyzed may be of help to depict underlying pathophysiological mechanisms. For the others 8 patients, LC/MS analysis of the CSF was normal. This can be explained by incomplete coverage of the whole metabolome. Furthermore, 22 of the 60 patients with unexplained encephalopathies presented at least one or more metabolites in an abnormally high concentration. Among these 22 patients, our LC/MS study enabled the grouping of 3 patients, given the high number of common abnormalities detected in their fingerprints. Interestingly, two of these patients have already been grouped from a previous NMR study in which a significant elevation of free sialic acid was detected exclusively in their CSF [5].

In conclusion, The CSF profile of abnormal metabolites might provide new insights into the pathogenic mechanisms of known diseases. In patients with unexplained encephalopathies, the precise delineation of individual biochemical phenotypes makes LC/MS based metabolomics of CSF a promising complementary tool to high throughput genetic approaches.

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Oral Session

Thursday, 20th September

Room B-1

15:00 – 17:00

Session 38: Mass Spectrometry for Metabolic Diseases

Chair: Makoto Yoshino (Kurume University, Japan)
Seiji Yamaguchi (Shimane University, Japan)

S38-1600

16:00 – 16:20

Highly sensitive and specific clinical diagnostics of lysosomal storage diseases using multiple reaction monitoring mass spectrometry, fluorimetry, and affinity-proteomics

Michael Przybylski[1], Claudia Cozma[1], Marius I Iurascu[1], Laura Ion[1], Adolf Muehl[2], Alina Petre[1,3], Michael L Gross[3], Stefan Maeser[2]

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Keywords:
LSDs ; diagnostics ; mass spectrometry, fluorimetry ;

Novel aspects:
Development of molecular clininical diagnostics for lysosomal storage diseases by MS-MRM and fluorimetry using identical substrates, suitable for multiplex diagnostics on dry blood spots.

Abstract:
Lysosomal storage disorders (LSDs) are a group of approximately 60-70 "rare " metabolic disorders caused by the deficiency of single lysosomal enzymes in metabolizing biopolymer substrates, or by a defect lysosomal membrane protein. If enzymes as a result of an inherited genetic defect or loss of function due to biochemical defects are out of function, (LSDs) are manifesting through a broad, unspecific spectrum of life-threatening clinical symptoms including bone, muscle and organ enlargement (e.g., heart, liver and spleen) , differing by disease, age of onset and severity. Most LSDs are inherited in an autosomal recessive manner ; however, *Fabry disease* and *Hunter syndrome* (MPSII) are X-linked disorders. For some LSDs, enzyme replacement therapies (ERT) have been developed and have been shown to be curative ; however, ERT is critical to start as soon as possible and therapy at late-stages is often no longer effective, thus rendering rapid and efficient diagnosis of key importance. Here we describe highly specific and sensitive diagnostics on dry blood spots (DBS) for (i) , molecular determinations of LSDs, particularly muco-polysaccaridoses and muco-lipidoses, by simultaneous fluorimetric and mass spectrometric analysis using newly developed, identical substrates and standard derivatives ; (ii) , clinical diagnostics of LSDs by multiplex- MS-MRM analysis using specific fragmentations of substrates and multiply-^{13}C-labelled standard derivatives ; (iii) , the clinical validation of the new methods with DBS samples, as shown for patients with established LSDs (Fabry ; MPS-I ; MPS-VI) . The new methods enable a substantial extension and improvement of current diagnostics, and provide access to a spectrum of LSDs not hitherto amenable to clinical diagnostics. Moreover, most recently methods have been developed for the direct determination of LSD proteins *in vivo* by affinity-proteomics and -mass spectrometry [1 - 3] , using epitope- specific antibodies.

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S38-1620
16:20 – 16:40

Mass spectrometry in newborn screening of fatty acid oxidation disorders - diagnostic approach using lymphocytes

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Keywords:
fatty acid oxidation, newborn screening

Novel aspects:
Labeled acylcarnitines in lymphocytes loaded with deuterium-labeled palmitate can be measured using tandem mass spectrometry, and are useful for quick and accurate diagnosis of fatty acid oxidation disorders.

Abstract:
[Background] Fatty acid oxidation disorders, as inborn errors of metabolism, are characterized by sudden infant death or acute encephalopathy due to hypoglycemia and hyperammonemia during fasting events, and are designated as important target disorders for newborn screening using tandem mass spectrometry (MS/MS), in which acylcarnitines in dried blood spots are measured. However, there are some difficulties in selecting optimum screening markers with adequate cut-off values, partly due to unique nature of disorders or lack of consistency in measurement among MS/MS instruments, which caused a considerable numbers of cases with false-positive results and even with false-negative results. Thus, enzyme assays and mutation analyses are mandatory for the diagnosis, although these tests are not convenient in clinical settings. Instead, we tried to analyze acylcarnitines in lymphocytes loaded with stable-isotope labeled fatty acid for quick diagnosis of fatty acid oxidation disorders.

[Methods] Heparinized whole blood samples (5 ml) were obtained from patients with very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD), carnitine palmitoyltransferase I deficiency (CPT 1 D), CPT 2 D, mitochondrial tri-functional protein deficiency (TFPD), and multiple acyl-CoA dehydrogenase deficiency (MADD). Lymphocytes, collected using Ficall-Paque solution, were suspended in 1.0 ml of Dulbecco's-PBS (D-PBS). The cell suspension was mixed with D-PBS containing fatty acid-free BSA, *l*-carnitine, and d_{31}-palmitate (d_{15}-caprylate, if necessary), and was incubated for 2 hrs. The washed lymphocytes were homogenized in methanol and centrifuged. Acylcarnitines in the supernatant, which was spiked with stable-isotope-labeled acylcarnitines as internal standards, were analyzed by flow-injection electrospray-ionization tandem mass spectrometry using a model API4000 LC/MS/MS system (AB Sciex). Precursor ion scanning using a product ion of m/z 85 was performed, and quantification was done using the peak heights of the respective acylcarnitines.

[Results& Discussion] In lymphocytes loaded with d_{31}-palmitic acid, a series of labeled acylcarnitines, from d_{31}-palmitoylcarnitine (d_{31}C16) to d_1-acetylcarnitine (d_1C 2), were observed, and the concentrations were determined. The amount ratios of d_1C 2 to d_{31}C16 (d_1C 2 /d_{31}C16) in patients with long-chain fatty acid oxidation disorders (VLCADD, CPT-II, TFPD) and MADD were decreased significantly as compared with those in controls, and were thought to be a useful marker to assess fatty acid oxidation ability. With regard to diagnosis of these disorders, metabolic blocks were assessed by the ratio of the accumulated labeled-acylcarnitine to the decreased one ; d_{31}C16/d_{27}C14 was elevated in CPT 2 D, d_{27}C14/d_{23}C12 in VLCADD, d_{29}C16OH/d_{31}C16 in TFPD, and d_{11}C 6 /d_1C 2 in patients with MADD. In lymphocytes of CPT 1 D, however, the concentrations of d_{31}C16 were decreased because of the deficient activity of CPT1. In the present study, lymphocytes collected from an aliquot of heparinized blood varied considerably, and the concentrations of labeled acylcarnitine derived from d_{31}-palmitate themselves were hardly evaluated. In contrast with palmitate, caprylate can be metabolized in lymphocytes of CPT 1 D by the other transferase than CPT1. We measured acylcarnitines in lymphocytes loaded with d_{31}-palmitate and d_{15}-caprylate, in addition, and found that, in CPT 1 D, the ratios of d_7C 4 /d_{31}C16 value in lymphocytes loaded with d_{31}-palmitate and d_{15}-caprylate to d_7C 4 /d_{31}C16 value in lymphocytes loaded with d_{31}-palmitatealone were increased as compared to the ratios in controls.

[Conclusion] Enzyme defects can be detected by the labeled-acylcarnitine ratios designated to the disease. The present test using lymphocytes loaded with labeled fatty acid is simple and quick, and useful in clinical settings.

Oral Session

Thursday, 20th September

Room B-1

15:00 – 17:00

Session 38: Mass Spectrometry for Metabolic Diseases

Chair: Makoto Yoshino (Kurume University, Japan)
Seiji Yamaguchi (Shimane University, Japan)

S38-1640

16:40 – 17:00

Application of in-vitro probe acylcarnitine assay using tandem mass spectrometry for the evaluation of mitochondrial fatty acid oxidation

Jamiyan Purevsuren, Hironori Kobayashi, Yuki Hasegawa, Kenji Yamada, Tomoo Takahashi, Seiji Yamaguchi

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Keywords:
acylcarnitine, fatty acid oxidation disorder

Novel aspects:
In vitro probe assay (IVP) was developed for diagnosis of primary carnitine deficiency and carnitine palmitoyltransferase-1 deficiency.IVP assay was applied for evaluation of fatty acid oxidation in various conditions.

Abstract:
Background: Fatty acid oxidation (FAO) disorder is a relatively common type of inherited metabolic disorders, and detected by blood acylcarnitine analysis using tandem mass spectrometry (TMS). In vitro probe acylcarnitine (IVP) assay with cultured cells and TMS is used for determination of FAO capacity to confirm diagnosis of FAO disorders. We applied the IVP assay to evaluate the effects of endogenous and exogenous factors such as heat stress, cytokines, bacterial toxins or some drugs on FAO capacity in the cells. Furthermore, we developed a strategy to make diagnosis of primary carnitine deficiency (PCD) or carnitine palmitpoyltransferase-1 (CPT1) deficiency using IVP assay, for which the IVP assay diagnosis has been difficult.
Materials and Methods: Fibroblasts from healthy controls and patients with medium chain acyl-CoA dehydrogenase (MCAD), very long chain acyl-CoA dehydrogenase (VLCAD), CPT-1 and CPT-2 deficiencies, glutaric acidemia type 2 (GA2) and PCD were examined for acylcarnitine (AC) profiles using in IVP assay. Acylcarnitine profiles were analyzed in various conditions, and intra- and extracellar lysates of fibroblasts. The followings are the modified conditions: a) various fatty acid substrates such as octanoic (C8) or palmitic acid (C16) loaded; b) various concentration of free L-carnitine (reduced (10 µmol/L), physiological (50 µmol/L) or excessive concentration (400µmol/L) loaded. Effect of endogenous factors, cytokines (IL-1β, IL-6, IL-10 and TNF-α) and/or exogenous bacterial toxin, cereulide, on FAO were analyzed.
Results and Discussions: In healthy control, acetylcarnitine (C2), which is a final product of fatty acid oxidation, was the only prominent metabolite when palmitic acid were loaded with excessive free L-carnitine. A substantial accumulation of specific ACs was observed in the culture medium of cells with MCAD, VLCAD, CPT-2 deficiencies and GA2. In this condition, however, AC profile in cell cultured medium was not specific for PCD and CPT-1 deficiency. It was revealed that the profile in intracellular lysates with low concentration of free L-carnitine was diagnostic for PCD and CPT-1 deficiency.
In the experiments with endogenous cytokines, AC profile in the presence of IL-6 and IL-10 was similar to those without cytokines in controls and patients with various FAODs. In the presence of IL-1β and TNF-α, however, C16 was significantly increased in CPT-2 deficiency, while C14 and C16 were elevated in VLCAD deficiency. Wide range ACs (C6-C16) were significantly increased in GA2, whereas ACs were barely affected in MCAD deficiency and controls.
In summary, the current IVP assay is a simple and powerful method to make an accurate diagnosis of FAO disorders, and also a good method to evaluate FAO capacity in various conditions.

Oral Session

Thursday, 20th September

Room D

15:00 – 17:00

Session 39: MS Informatics for Quantitation

Chair: David Fenyö (New York University, USA)

S39-1500

15:00 – 15:40

[Keynote Lecture] Detection of differential expression of splice variants in the MaxQuant framework for quantitative proteomics

Jürgen Cox[1,2], Nagarjuna Nagaraj[1,2], Cornelia Schoenbauer[1,2], Frank Schnorrer[1,2], Matthias Mann[1,2]

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Keywords:
Quantification, alternative splicing

Novel aspects:
Proteome-scale automated detection of quantitative differences between proteins from differentially spliced transcripts allows distinguishing functionally redundant from functionally distinct isoforms.

Abstract:
Mass spectrometry based quantitative proteomics is becoming more comprehensive in terms of providing protein expression values or changes in expression for the majority of proteins expressed in a cell. However, in most cases only a summary quantification of all the protein isoforms originating from the same gene location is available. Biologists know many cases in which the specific function of a gene is carried out by a particular splice variant, while other products from the same gene do not have the same functional capabilities. Therefore is desirable in general to perform protein quantification on peptides derived from exons or splice junctions that are specific to a certain spliced transcript.

Label-free or labeling-based peptide quantification is carried out with the MaxQuant software. A new MaxQuant module maps the identified peptides to the ENSEMBL genome including to annotated splice variants. We define and apply a statistical test designed to detect genes with significant differential regulation between at least two isoforms over two or multiple sample groups. While the basic form of the test requires at least two peptides specific for an isoform, a modified version of the test allows in some cases for detection of splice variants that are distinguished by a single peptide.

Several high-resolution LC-MS data sets obtained with different Thermo Fisher Orbitrap platforms were obtained including the Q Exactive and Velos platforms. Label-free as well as SILAC labeled data was included. The data originates from multiple different species, including human, mouse and Drosophila. Raw data was processed with MaxQuant in the standard manner. The quantitative peptide data was further processed with the Perseus module which is dedicated to the statistical analysis and systems biology of proteome and other high-throughput data. The resulting normalized quantitative peptide data was further analyzed with the newly developed splice-variant specific quantification and genome mapping module of MaxQuant. We detected hundreds of cases where different splice variants have significantly different quantitative profiles over the measured samples, implying different regulation and different biological functions. Several positive controls were detected where the MaxQuant output agrees with prior biological knowledge and many as yet unknown cases of differential splice variant regulation are found. Comparison with deep sequencing data of the transcriptome reveals many cases where the transcriptome and proteome data agree. In addition, specific cases of disagreement between transcriptomics and proteomics data were clearly evident. We analyze data from a quantitative study of 28 different mouse tissues and discuss particular examples of correlating and non- or anti-correlating slice variants. Furthermore we investigate if there are significant differences between isoforms originating from the same gene and isoforms encoded in different genomic locations. Finally, we applied our framework to functionally distinct muscle types in Drosophila. Preliminary results indicated striking differences in the structural sarcomeric components, for instance between leg muscles and flight muscles. These results are important to understand tissue specific transcriptional regulation and differential tissue specific splicing. They would have been very difficult to discover with traditional targeted approaches such as immuno detection.

Oral Session

Thursday, 20th September

Room D

15:00 – 17:00

Session 39: MS Informatics for Quantitation

Chair: David Fenyö (New York University, USA)

S39-1540 Peptide Yields Depend on Digestion Conditions

15:40 – 16:00

Stein E Stephen, Yuxue Liang, Eric Yan
National Institute of Standards and technology, Gaithersburg, MD, USA

Keywords:
protein digestion, proteomics, variability, qa/qc

Novel aspects:
A quantitative determination of variations in peptide yields in a tryptic digestion for individual proteins is presented using digests of N15-labeled proteins as internal standards.

Abstract:
Introduction : Quantitative proteomics is based on the assumption that concentrations of proteins are proportional to their derived peptides. Therefore, changes in relative yields of peptides from a protein will cause apparent changes in concentrations of the proteins themselves. We examine these changes by measuring relative peptide yields from tryptic digests of two different plasma proteins under various digestion conditions using spiked-in isotopically-labeled mixtures peptides from digests of the corresponding N15-labeled proteins.
Method : The proteins examined were human serum albumin and apoliproptein A1. They were each digested in trypsin for 2 and 18 hours using up to 12 different digestion conditions. Denaturants included urea, guanidine (at low and high temperature) , methanol, trifluoroethanol, rapigest and heat alone. Proteomics grade (alkylated) trypsin was generally used, but some runs employed non-alkylated trypsin and Lys-C. Time course digestion studies were also done over 15 min. to 18 hours. Analysis was done with a Velos Orbitrap by monitoring relative MS 1 signals of labeled and unlabeled peptides, as identified by MS 2 analysis using in-house software. Aliquots of N15-digest were added to digests dpme under varying conditions immediately after quenching the digestions relative concentrations of the labeled peptides were therefore the same in each digest. Moreover, initial proteins concentrations were identical since proteins came from a single protein solution. Relative peptide yields of over one hundred peptide ions were determined for each protein. Peptides included simple tryptic, missed cleavage, semitryptic and a variety of modifications. Measurement accuracy was better than 5 % as determined by comparing different charge states of the same peptide, which should have the same relative concentrations of N15 peptides.
Results : Significant variations in yields of most peptides from trypsin digestion from the same protein were found with changing digestion conditions. Some variations clearly result from different different typsin activities or digestion times. But most could not be explained so simply. Almost all peptides varied at least a factor of two for the different digestion conditions, even when digested for the same time period many varied far more. At the current stage of analysis, this complex behavior cannot be expressed in rules, but appears to be a consequence of a variety of hard-to-separate factors including peptide release rates, solubility and chemical reactivity. One trend did seem clear, variations increased with increasing peptide length. Furthermore, digest-to-digest reproducibility was not high, indicating that a great deal of attention will have to be made to ensure sample-to-sample consistency of peptide yields even using the same materials and methods. Other implications of these results for quantitative proteomics are discussed.
Conclusion : Yields of tryptic peptides from proteins are variable and depend on both the method and peptide in a way that cannot presently be predicted. Without specific validation, concentrations of 'proteotypic' peptides cannot be assumed to be equal to their precursor proteins.

Oral Session

Thursday, 20th September

Room D

15:00 – 17:00

Session 39: MS Informatics for Quantitation

Chair: David Fenyö (New York University, USA)

S39-1600

16:00 – 16:20

Quantitative Proteomics of Embryonic Stem Cells Differentiation to Cardiomyocytes

Ekaterina Mostovenko, André M Deelder, Christine L Mummery, Robert Passier, Magnus Palmblad

Leiden University Medical Center, Leiden, Netherlands

Keywords:
label-free, quantitative proteomics, stem cell differentiation, scientific workflows

Novel aspects:
New, high-throughput quantitative proteomics methodology applied in the investigation of protein expression and localization during stem cell differentiation

Abstract:
Introduction

The pluripotency of embryonic stem cells (ESC) was demonstrated in mice in 1981 and much work has since been undertaken to create from them mature (fully differentiated), specialized somatic cells. In recent years, Mummery *et al.* successfully differentiated human ESC (hESC) into cardiomyocytes using different growth factors, including activin A and BMP 4. Resulting cell aggregates beat spontaneously and have a direct electrophysiological comparison with primary human fetal cardiomyocytes in culture. Although we are now able to influence the development of stem cells, many details in the differentiation process are still unclear. A better understanding of stem cell differentiation may lead to a better control of the differentiation process and to successful myocardium regeneration therapies. The aim of this work was to follow in detail, changes in protein expression and location during differentiation of hESCs into cardiomyocytes using label-free quantitative proteomics. Specifically, we relied on a recently developed and implemented FTICR-ion trap cluster designed for high throughput label-free quantitative proteomics to measure protein concentrations in many time points and biological replicates.

Methods
To obtain various stages of differentiating cardiomyocytes, hESCs were resuspended in defined differentiation medium containing BPEL medium and growth factors BMP4, ActivinA, VEGF and SCF for the first three days in 96-well plates. Cells were collected after 0 (undifferentiated hESC), 3, 7 and 15 days of incubation. The medium was removed, cells washed twice with warm (37°C) PBS and transferred to Eppendorf tubes. The cells were then lysed in 1 % SDS in a hot (70 °C) ultrasonic bath (VWR Ultrasonic Cleaner) for 15 min. Thirty µg of each lysate was loaded on a 10-lane 4-12% NuPAGE(R) Bis-Tris gel (Invitrogen, Carlsbad, CA,USA). From each lane 48 slices were cut out using a custom-made cutter (The Gel Company, San Francisco, CA, USA) and digested in gel and then analysed using LC-MS/MS using splitless parallel NanoLC-Ultra 2D plus ultra-high pressure liquid chromatography systems (Eksigent, Dublin, CA) coupled on-line to amaZon ETD high-capacity 3D ion traps (Bruker Daltonics, Bremen,Germany). Each time point and replicate was then analyzed on a 12 tesla solariX FTICR (Bruker) using the same type of column, but without any preceding gel fractionation. The acquired data was converted to mzXML by compassXport and processed by a scientific workflow in Taverna workbench, the core of the workflow derived from the Trans-Proteomic Pipeline. In the workflow, first the data from the ion traps were searched with X!Tandem and SpectraST for peptide identifications which were then combined by iProphet and assigned with accurate masses and ion intensities from the FTICR.

Results
We obtained a wide range of peptide identifications (~40,000 peptides, 5,200 proteins) for further use in combination with accurate and quantitative FTICR data to generate protein expression profiles from 15 time point (sampling each day of incubation) and replicates. Gene ontology analysis revealed that sample preparation is robust and reproducible with respect to the relative yields of membrane (22%), intracellular (42%), extracellular (10%) and nuclear proteins (26%), with the number of proteins identified in different cellular compartments being consistent between samples. The difference in protein expression profiles increased as a function of differentiation stage. From 2,470 proteins presented in all samples, as expected, proteins involved in motor and channel transporter activities increased in relative abundance in later time points, when cells become differentiated cardiomyocytes.
All steps of the data handling (from converting raw files to mzXML all the way to graphical visualization) were piped in one workflow using Taverna. Such automated analysis led to an easier data processing and allowed us to deal with large datasets.

Oral Session

Thursday, 20th September

Room D

15:00 – 17:00

Session 39: MS Informatics for Quantitation

Chair: David Fenyö (New York University, USA)

S39-1620

16:20 – 16:40

Estimation of relative protein abundance and statistical analysis of proteomic data from multiple iTRAQ experiments, to assess micronutrient deficiencies.

Ingo Ruczinski, Keith P West, Robert N Cole, Kerry Schulze, Shelley Herbrich, Parul Christian, Jim D Yager, John D Groopman

Johns Hopkins University, Baltimore MD, USA

Keywords:
iTRAQ, statistical models, micronutrient deficiency

Novel aspects:
Development of novel robust, scalable, and efficient methods for relative protein abundance estimation and statistical inference, applied to a chronically undernourished child population in South Asia.

Abstract:
Introduction

We investigate strategies to estimate relative protein abundance from multiple iTRAQ experiments. We show that using the available biological data instead of masterpools for normalization within and across experiments yields more efficient estimators. We show how previously proposed linear mixed effects models can be improved and simplified for inference in case-control studies and studies with quantitative outcome, producing simple, efficient, and scalable approaches to detect proteins associated with the outcome of interest. We illustrate our methods using data from replicate iTRAQ experiments on samples of pooled serum, and from a large project carried out to define the nutriproteome (proteins in circulation that covary with nutrient concentrations or respond to nutritional exposures) in a young, chronically undernourished child population in South Asia.

Methods

8-plex stable isotope masstags (iTRAQ) were employed to identify and quantify serum proteins of 400 Nepalese children by high-throughput tandem mass spectrometry. Isotopically resolved masses in MS and MS/MS spectra were extracted with and without deconvolution using Thermo Scientific Xtract software. Both data sets were searched against the RefSeq 40 database using Mascot (Matrix Science) through Proteome Discoverer software (v1.3, Thermo Scientific). Peptide identifications from Mascot searches were processed within the Proteome Discoverer to identify peptides with a confidence threshold 1% False Discovery Rate (FDR), based on a concatenated decoy database search. Peptide identifications with the highest mascot score from the same peptide matched spectrum before and after deconvolution by the Xtract function were used for quantitation of reporter ion intensities.

Results

A micronutrient deficient population of early school aged children, typical of the Terai of Nepal, has been established using 20 nutrient-indicators (including Vitamins A, B6, B12, copper, iron and zinc) and indicators of inflammation (including CRP), along with their health profile and associated plasma proteome. With a 1% false discovery rate, over 4600 proteins have been so far identified in 400 child plasma samples at least once, reflecting the breadth of detection that is possible within the dynamic range reached in this project. The vast majority of protein identifications, however, are based on single peptides and have been identified in small numbers of children. Nonetheless, 140 proteins have so far been identified and quantified in every child, and an additional 160 proteins have been quantified in more than 70% of all children.

Novel methods to estimate protein relative abundances were employed, accounting for dependencies of reporter ion intensities within peptides, and peptides within protein. Newly devised linear mixed effects models were used to assess protein-nutrient relationships, accounting for between experiment variability. We demonstrate that these methods provide a robust, scalable, and more efficient alternative for statistical inference. For each nutrient indicator in our case study we assessed the nutriproteome of correlated proteins (p-values < 0.05 and/or q-values < 0.10), generating lists in excess of 100 proteins for some of the nutrients, with very strong relationships emerging for many protein-nutrient combinations (some $R^2 > 0.75$, $p < 1e\text{-}100$). We also defined protein "signatures" (linear combinations of relative protein abundances) with predictive power for micronutrient status.

Oral Session

Thursday, 20th September

Room D

15:00 – 17:00

Session 39: MS Informatics for Quantitation

Chair: David Fenyö (New York University, USA)

S39-1640

16:40 – 17:00

Accurate mass quantitation of in vivo plasma samples using high resolution QTof and MSE data analysis across a global network

McDonald Stephen[1], Mark Wrona[1], Julie Laterreur[2], Nigel Ewing[3], Eric Lanlois[2], Joanne Mather[1], Debadeep Bhattacharya[1], Robert S Plumb[1]

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Keywords:
DMPK, Bioanalysis, Metabolite Identification, Exact Mass, Quan/Qual

Novel aspects:
Unbiased, MSE methodology for obtaining in vivo high resolution quantitative datasets using UPLC-Qtof platforms in a collaborative environment.

Abstract:
The current realm of drug metabolism has been challenged with dealing with ever increasing need for sensitivity, selectivity and resolution. As instrument platforms have evolved to address and often exceed these basic tenets, laboratories are also beginning to look for additional ways to use the data more intelligently. QTof platforms equipped with MS^E data analysis offer a balance of scan speed, sensitivity and resolution have the ability to both provide rich qualitative datasets and put the data into context (the quantitative aspect). This presentation focuses on the analysis of *in vivo* plasma PK with multiple lab, company, site, and country variables on the Quant of platform generating datasets that can be used for both qualitative and more importantly quantitative decision making.

Pharmaceutical compound (Propranolol) was dosed in rats at 75 mg/kg. Samples were protein precipitated, using a 2 : 1 acetonitrile (containing IS) followed by centrifugation. Samples were frozen (-80 ° C) and shipped to several locations. Samples were analyzed on four different Acquity -Xevo Qtof UPLC-MS systems across two companies, four different sites in three different geographies (Canada, USA and the United Kingdom). PK curves for animals were reported with levels of ~0.5ng/mL to 1000 ng/mL (as measured by traditional QqQ approach), a difference of ~ 4 orders of dynamic range in the presence of plasma matrix background. Results presented will show the accuracy and precision (with standard curves and QCs) with which these analyses were performed and the benefits of such an approach.

All Xevo G 2 -QTofs were operated in positive ESI resolution mode with a minimum measured resolution of 25,000 (FWHM at m/z 520) with an analysis mass range of m/z 50 to 1200 running at 10Hz. With MS^E enabled to facilitate collection of both intact precursor full scan data (for quantitative analysis and screening) and generate fragmentation data for all precursors (high collision energy, ramping from 10 to 50eV), samples were effectively being scanned at 5 scans per second. The average chromatographic peak width observed across the sites was 3 seconds, and this typically yielded a minimum of 10 points for both identified intact precursors and internal standard (used for quantitation) as well as 10 points of fragment ion data (which is then available for qualitative analysis and confirmation of putative metabolites). Rat plasma from two rats, collected from 0 -24hrs were pooled (this allowed more material to provide additional injections for analytical reproducibility, rather than measure biological variability which is assumed to exist). Pooled propranolol samples were quenched with acetonitrile containing propranolol-d 7 to provide reliable and accurate quantitation of the dataset. Samples analysis showed extremely consistent injection reproducibility, obtaining R^2 values of >0.95 across several orders of dynamic range in the presence of complex matrix.
The methodology demonstrates the ability to capture a broadly applicable data set well suited for quick analyses where both quantitative and qualitative data find utility in the drug metabolism environment.
This need for maximizing knowledge obtained from each analysis, management of the data itself, the ability to share this data with scientific departments and leveraging expertise distributed, across sites, across companies and indeed across oceans is a continuing theme across the field of drug discovery. This presentation will show new ways to conduct experiments that increase the capabilities of modern DMPK laboratories.

Oral Session

Thursday, 20th September

Room E

15:00 – 17:00

Session 40: Environment II

Chair: Takeshi Nakano (Osaka University, Japan)

S40-1500

15:00 – 15:40

[Keynote Lecture] Powerful GC-ToF-MS Techniques for Quantification of Legacy Pollutants and Screening and Identification of Emerging Pollutants

Peter S Haglund, Conny Danielsson, Mikael Harju, Patricia Moreira-Bastos, Ulrika Olofsson

Umeå University, Umeå, Sweden

Keywords:
GC×GC, multi-reflectron ToF, PCBs, dioxins, emerging pollutants

Novel aspects:
Two-dimensional gas chromatography and ultra high-resolution mass spectrometry provide exceptional peak capacity, mass resolution and mass precision and open new possibilities in environmental analyses.

Abstract:
Environmental samples often present a challenging task to the analytical chemists. The target compounds are generally present at trace levels in a complex cocktail of natural and anthropogenic compounds. In order to accurately quantify such trace compounds it is essential to reduce the background, but also to efficiently separate the target compounds from each other and residual matrix. Although some advances have been made in the streamlining of sample preparation, e.g. using simultaneous extraction and clean-up, the focus on this talk will be on powerful GC-MS methods for quantitative and qualitative analysis of legacy and emerging pollutants.

Over the last decades, comprehensive two-dimensional GC (GC×GC) has evolved into a robust and exceptionally powerful technique for group-type as well as within-group (isomer) separations. Group-type separations of petroleum hydrocarbons were one of the first and most important areas of application. In the case of isomer separations, one of the more demanding tasks is the complete separation of the 209 PCBs or 210 polychlorinated dioxins and furans (PCDD/Fs). Although the quest still continues, it has been possible to greatly enhance the separation. It is now possible to separate all dioxin-like PCBs from each other and from other PCBs, and separate all 2,3,7,8-PCDD/Fs from each other and from most other PCDD/Fs. It is also possible to perform enantioselective analysis of PCB atropisomers (asymmetric tri- and tetra-*ortho* PCBs). However, careful selection and optimization of the column sets are essential. For PCB and PCDD/F analysis the coupling of a long efficient non-polar first and a shape-selective liquid crystal second dimension column proved to be most successful; while for the atropisomers, the first column had to be changed to a permethylated cyclodextrin column. In these separations, the target compounds were more and less retained, respectively, by the liquid crystal phase than the potential interferences.

The high peak capacity of GC×GC ToF MS can also be utilized to perform comprehensive characterization of complex samples, e.g. extracts of soil from contaminated sites or water from sewage treatment plants (STPs). However, the number of components detected and tentatively identified in such non-target analyses are overwhelming and logical and efficient prioritization tools are required to make such studies feasible. Recently, two different approaches were tested: a risk-based prioritization and a property-based classification. In the first, quantitative structure-activity relationships were applied to all tentatively identified compounds in contaminated soil extracts and the measured concentrations were compared to the estimated no-effect levels to obtain a risk-ratio. All compounds with high risk ratios were prioritized for confirmatory analysis and, if confirmed, further analyzed in biota potentially affected by soil contaminants. The property based classification was also based on ration calculations, i.e. the concentration ratio of STP effluent and influent water. Compounds with ratios much lower than 1 are removed to a great extend, those with a ratio close to 1 are poorly removed, and those with a ratio above may actually be formed in the treatment process. In this way, compounds that are classified as poorly removed or metabolites may be identified, which will aid in the improvement of existing and design of new STP processes.

Although ultimate confirmation requires authentic standards novel GC-MS techniques such as ultra-high resolution ToF-MS may be used to provide valuable information. Such techniques deliver full-scan spectra at a resolution up to 50,000 and a mass precision of 1 ppm, which is sufficient to unambiguously determine elemental compositions of ions up to ca 150 amu (may be extended to ca. 500 amu using isotope abundance analysis). This is generally sufficient to confirm or reject tentative structures and greatly enhances the possibility to perform a correct manual interpretation.

Oral Session

Thursday, 20th September

Room E

15:00 – 17:00

Session 40: Environment II

Chair: Takeshi Nakano (Osaka University, Japan)

S40-1540 Dioxin Food Crises and New POPs: Challenges in Analyses

15:40 – 16:00

Jef Focant

University of Liege, Liege, Belgium

Keywords:
Food ; Screening ; SectorHRMS ; HRTOFMS ; GCxGC

Novel aspects:
Comprehensive emerging POP analyses

Abstract:
When more than a million of broiler chickens suddenly and unexpectedly died in the eastern and midwestern parts of the United States in late 1957, the first dioxin crisis record was set. The 1999 Belgian dioxin chicken gate affair ultimately demonstrated the economic damage such a contamination episode could yield to and pushed the European Union (EU) to set an efficient and pro-active monitoring program to ensure proper quality of the European food and feed web and try to maintain most of the population below tolerable weekly intake. The current European Commission (EC) strategy relies on the implementation of maximum (and action) residue levels (MRLs) for selected polychlorinated dibenzo-p-dioxins (PCDDs) , polychlorinated dibenzofurans (PCDFs) (both families actually called 'dioxins') , and dioxin-like polychlorinated biphenyls (DL-PCBs) .

To ensure the adequate production of comprehensive and reliable data on the presence of PCDD/Fs dioxins and DL-PCBs in food and feed, a screening-confirmatory approach was thus early adopted for the official control of the PCDD/F dioxins and DL-PCBs. In practice, screening is most of the time performed using chemical activated luciferase gene expression (CALUX) bioassays, response-binding assays (RBAs) based on the aryl hydrocarbon receptor (AhR) , although the sole confirmatory method is gas chromatography coupled to ^{13}C-labeled isotope dilution sector high resolution mass spectrometry (GC-IDHRMS) [1] . More than 10 years after the implementation of the screening-confirmatory approach, the analytical situation has drastically evolved because of advances in automation and hyphenation of parallel sample preparation techniques that allowed both cost and result delivery time to be significantly reduced (down to 350 EUR and 24h, respectively) for the confirmatory GC-IDHRMS method.

Nevertheless, RBAs could and should now be used with non-restrictive sample preparation techniques that would allow most toxicants present in the sample to interact with the AhR to give a general persistent organic pollutant (POP) toxicity information rather than an estimation limited to dioxin regulation compliance. This would be much more biologically relevant and would allow to enlarge current food safety practices to other known and unknown POPs. Sample showing high response for the biological screening should be further analyzed in the hope of identifying new compounds. The extension of the list of target compounds to more 'exotic' (un) suspected persistent molecules present in our food requires both chromatographic resolution and instrumental limits of detection (iLODs) to be improved.

Using comprehensive two-dimensional GC (GCxGC) [2] or cryogenic zone compression (CZC) [3] GC coupled to HR time-of-flight MS (HRTOFMS) operating in full scan (FS) mode [4] could nicely complement the classical GC-sector IDHRMS performing in selected ion monitoring (SIM) used for target analyses. This would open the possibility to screen for other compounds (organochlorine pesticides, halogenated flame retardants, GC-amenable perfluorinated compounds,) than the one under current regulation without compromising sensitivity. Recent advances in coupling between GCxGC and HRTOFMS nicely put this approach one step further as it allows to produce elemental composition data for unknown compounds present in complex mixtures. Furthermore, when considering halogenated compounds, such a system can be granted by a tremendous improvement in sensitivity (back to low fg iLODs) by favoring resonance electron capture (REC) when operating in negative chemical ionization (NCI) rather than electron impact (EI) . Major recent analytical advances in the GC-MS of emerging GC-amenable POPs in the context of prioritization of new targets for food control will be highlighted.

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Oral Session

Thursday, 20th September

Room E

15:00 – 17:00

Session 40: Environment II

Chair: Takeshi Nakano (Osaka University, Japan)

S40-1600

16:00 – 16:20

New Perspectives in the Mass Spectroscopy Determination of Dioxin-like Substances in Environmental and Food Samples

Angel Garcia-Bermejo[2], Manuela Abalos[1], Lisa Mattioli[1], Maria Jose Gonzalez[2], Belen Gomara[2], Esteban Abad[1]

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Keywords:
dioxin-like substances, triple-quadrupole, GC-QqQ (MS/MS), HRGC-HRMS

Novel aspects:
A novel approach for the analysis of dioxin-like substances in environmental and food samples based on the use of GC-QqQ (MS/MS) is compared to reference techniques based on HRMS.

Abstract:
Due to an increased concern of the dietary exposure of dioxin-like compounds to the general public, the accurate determination of these compounds has become of great interest. These compounds are found in the environment and food and feed matrices at very low levels therefore analytical techniques and instrumentation providing precision, sensitivity and adequate limits of detection are necessary.

In the past dioxin-like compounds have been analyzed with gas chromatography coupled to single quadrupole and ion trap mass spectrometry, two-dimensional gas chromatography coupled to micro electron capture detection, and time-of-flight mass spectrometry. These methods are alternatives to the current reference instrumentation of high resolution gas chromatography high resolution mass spectrometry (HRGC-HRMS) for the analysis of many persistent organic pollutants, mainly dioxin-like compounds. In general, dioxins are analyzed at 10,000 resolution, ionized with a positive electron impact (EI+) source and quantified based on the isotope-dilution method. Magnetic sector remains the choice instrument for the analysis of dioxins as it allows for the necessary sensitivity at very low levels and the high resolution to separate the peaks. Although HRGC-HRMS is commonly used to analyze for dioxin-like compounds, this instrumentation is costly and high maintenance therefore alternative instrumentation are continuously under development[1]. Gas chromatography coupled to tandem mass spectrometry with a triple quadrupole analyzer (GC-QqQ (MS/MS)) seems to potentially have the sensitivity and selectivity compared to HRGC-HRMS[2,3]. Transitions from different precursors to product ions have been evaluated at several collision energies in order to determine the optimal conditions for the analysis of PCDD/Fs and DL-PCBs by GC-QqQ (MS/MS). Results are compared to those obtained by GC-HRMS in order to assess the applicability of this technique for the analysis of dioxins in environmental and food samples.

Comparative analyses were performed on a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) equipped with a triple quadrupole (TSQ Quantum XLS, Thermo Fisher Scientific, Bremen, Germany) and on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a DFS high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) controlled by an Xcalibur data system.

For optimization of the multiple reaction monitoring (MRM) method, different transitions were studied in order to select the most intense and, if possible, to achieve the highest number of identification points. For PCDD/Fs, the loss of the COCl group was the most abundant when collision induced dissociation (CID) voltages around 25 and 30 V were applied. Collision energies were always higher for PCDFs. In addition, other transitions were observed and studied when CID was set to higher values (40-50 V). Similar behaviour was observed for DL-PCBs, with the most intense transitions from the molecular cluster to the loss of two chlorine atoms for the congeners investigated.

Acknowledgements

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Oral Session

Thursday, 20th September

Room E

15:00 – 17:00

Session 40: Environment II

Chair: Takeshi Nakano (Osaka University, Japan)

S40-1620 GC-MS studies on POPs in Brazilian Ecosystems

16:20 – 16:40

Joao Paulo M Torres[1], Jose Lailson-Brito[2], Paulo R Dorneles[1], Rodrigo O Meire[1], Larissa S Cunha[1], Daniele Botaro[1], Giselle C Saldanha[3], Claudio E Azevedo e Silva[1], Dayse Aline M Rocha[1], Olaf Malm[1], Karl W Schramm[4], Begona Jimenez[5]

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Keywords:
GC-MS, POPs, PCBs, OCPs, PCDD/PCDFs

Novel aspects:
Using GC-MS, Hot Spots of POPs of Pollution were found in the Amazon, on fishes from tropical rivers and in Coastal Zones of Brazil

Abstract:
Environmental contamination by POPs where investigated in the biota of different Brazilian ecosystems, including the Amazon equatorial forest in the northern part of the country, tropical river basins, coastal zones and some enclosed bays located near densely populated areas. In the Amazon and on tropical river basins, we have focused our work on different kinds of fishes that represent the main protein source for the local population. In costal zones, we collected samples of several species of marine mammals (e.g. : dolphins and porpoises) as well as one species of tuna (Thunnus albacares) , one piscivorous bird species (Sula leucogaster) sampled from 3 (three) different archipelagos and two species of bivalves (Perna perna and Nodipecten nodosus) obtained from aquaculture established inside coastal bays and lagoons. Most of this work was done in cooperation with foreign laboratories under academic cooperation and joint research projects where the fat extracts obtained with soxhlet apparatus and/ or pressurized automatic extraction devices (e.g. : Dionex or FMS) . After clean-up using acidic silica gel and florisil columns, the main equipment used in these laboratories was the high resolution gas chromatography, operating in NCI (Negative Chemical Ionization) mode and coupled to low resolution mass spectrometer (e.g. : Quadrupole) . Among several OCPs, PCBs and the pesticide DDT and its main metabolite DDE were found in all of the edible fish tissues (e.g. : Prochilodus sp.) samples analyzed in the Amazon and in the tropical rivers may represent a health risk to the local population. Levels of POPs pesticides and PCBs are also present in hair samples taken from this population. Regarding marine mammals, we could identify in coastal dolphins the presence of high levels of POPs on the blubber of the animals, including a whole bunch of organochlorinated pesticides, brominated flame retardants (e.g. : PBDEs) and also moderate levels of dioxins and furans, and in this case, a high resolution mass spectrometer ("magnetic sector ") equipment was used. The overall pattern of contamination is so clear and separate quite well 3 (three) different bays that we can even say the place where the animal lives, only by looking to it ´ s POPs results. A pelagic animal tends to have less contamination than coastal animals. Taking in account the work we did on the avian predatory specie, their eggs are also very contaminated near Rio de Janeiro city, with reducing concentrations in birds eggs sampled far from the main land. On the other hand, ocean pelagic fishes like tuna as well as the cultivated bivalves seems not to concentrated high levels of pollutants in their edible tissues, even if they grown in highly impacted ecosystems. This work represents part of the effort and the commitment of different scientists from different Universities in Brazil to keep tuned with the utmost development of this field of work and to produce high quality data in order to help in the protection of tropical environments. Taking this in account, another application of these mass spectrometer techniques by our group is the deposition of legacy and current-use pesticide on the top of mountains and plateau areas, we found that DDT, PCBs, dioxin/furans, several POPs pesticides as well as endosulfan and chlorpyrifos residues are being deposited over these tropical mountains that are mostly located on natural parks. Nowadays, since cattle grazing activities are present in these areas, in order to cope to the GAPs project and also in order to respond to official demands on scientific results, we are presently collecting raw milk from these areas to see if this contamination is also being transmitted to the food chain. Acknowledgements : CNPq, CAPES, FAPERJ, UERJ and UFRJ for invaluable support on our research activities.

Oral Session

Thursday, 20th September

Room E

15:00 – 17:00

Session 40: Environment II

Chair: Takeshi Nakano (Osaka University, Japan)

S40-1640

16:40 – 17:00

Structural Analysis of Cyclodextrin Inclusion Complexes Formed in Nonpolar Solvents

Toshiyuki Kida, Haruyasu Asahara, Takuya Iwamoto, Mitsuru Akashi

Osaka University, Suita, Japan

Keywords:
cyclodextrin, inclusion complex, nonpolar solvent, structure

Novel aspects:
This is the first report on the structural analysis of cyclodextrin inclusion complexes formed in nonpolar media.

Abstract:
Cyclodextrins (CDs) have played a crucial role in various fields including supramolecular chemistry and analytical science due to their unique properties to form inclusion complexes with a great variety of molecules. However, inclusion complex formation with CDs has been limited to aqueous media and several kinds of polar organic media. On the other hand, the effective guest inclusion into the CD caviry in nonpolar media has not been achieved yet, possibly because the main driving force for the guest inclusion is hydrophobic interactions and/or van der Waals interactions between the guests and the CD cavity, and thus the enormous amounts of nonpolar solvents become a strong competitor for inclusion within the CD cavity.Recently, we found that 6-*O*-modifiedbeta-CD formed inclusion complexes with polychlorinated aromatic compounds in nonpolar media including benzene and cyclohexane, possibly through the dipole-dipole interactions between the CD cavity and the guest molecule, as well as the spatial fit of the guest into the CD cavity. In this presentation, we report the inclusion complex formation between 6-*O*-modifiedbeta-CD and various aromatic guests in nonpolar media, and the structural analysis of the resulting inclusion complexes by mass spectrometry.
Heptakis (6-*O*-triisopropylsilyl) -beta-CD (TIPS-beta-CD) and heptakis (6-*O*-*tert*-butyldimethylsilyl) -beta-CD (TBDMS-beta-CD) were used as CD hosts. Inclusion complex formation between the CD hosts and the aromatic guests in nonpolar solvents were evaluated by NMR spectroscopy. The association constants between the CD hosts and the aromatic guests were determined by ^{1}H-NMR titration method. These CDs effectively formed 2 : 1 inclusion complexes with pyrene in benzene-d_6 and cyclohexane-d_{12} with considerably high association constants. The 2 : 1 inclusion complex formation between TIPS-beta-CD and pyrene in nonpolar solvents was also confirmed by the APPI-MS analysis. Crystallographic study of the TIPS-beta-CD/pyrene inclusion complex obtained from the benzene solution showed the formation of a unique ternary complex among TIPS-beta-CD, pyrene, and the solvent. Interestingly, the pyrene molecule forms a sandwich-type complex with two benzene molecules through pi-pi interactions, and is located at the center of the dimer cavity. The crystalline structure suggested that the interaction between the solvent and pyrene affects the mode of pyrene inclusion within the CD dimer cavity.

PTh-001
11:10 – 12:20

Development, Evaluation and Comparison of Calibration Standards for MALDI-MS in the > 100 kDa m/z Range

Simon Weidmann[1], Konstantin Barylyuk[1], Stefanie Maedler[1,2], Renato Zenobi[1]

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Keywords:
high-mass MALDI, calibration, concatenated proteins

Novel aspects:
The use of recombinantely expressed concatenated proteins is a excellent possibility for calibration in high-mass MALDI-MS. The masses of the calibrants are well known and span a wide m/z range.

Abstract:
Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a very versatile tool for studying protein-protein or protein-DNA complexes and for the determination of their stoichiometry and interaction sites. The use of MALDI-MS for such purposes has certain advantages over electrospray ionization (ESI) MS, since the spectra obtained consist of mainly low charge states, which yield direct information about mass and stoichiometry of the sample under investigation. However, the low charge states of the ions also lead to high m/z ratios, generally well above 100 kDa. Up to now, no calibrants are commercially available for such a high m/z range. A suitable calibrant needs to fulfill some requirements : it must have a high and well-known mass and should be detectable with good sensitivity and high resolution. The m/z range that is covered by the calibrant or its multimers should be as wide as possible. Extension of the calibration range combining standards from different substance classes is possible, but should be avoided, since such combinations lead to signal suppression caused by different ionization properties and thus the handling of the calibration system becomes difficult.

Potential candidates that fulfill these requirements are proteins, which have either monomeric subunits with high masses or form nonspecific multimers or complexes over a wide m/z range. Several possible calibrants were investigated including bovine thyroglobulin, a homodimeric protein with a total molecular weight of 660 kDa, protein histidine phosphatase, and GroEL. These two proteins form nonspecific multimers up to the decamer or pentamer, respectively. Evaluation of these candidates showed that either the mass range covered was not wide enough or that the signals were too broad to be useful.

To obtain a new calibration standard a protein expression vector was genetically engineered and expressed in E. coli. This vector consists of three concatenated copies of the maltodextrose binding protein (MBP) encoding gene and allows for the expression of MBP_3. The system is designed as such that the concatemer MBP_3 can be cleaved into MBP and MBP_2 subunits. Furthermore, a specific interaction site is implemented to allow for the selective covalent dimerization of MBP_2 or MBP_3 via a disulfide bond. Since the primary sequence is well-known, the observed signals can directly be used as calibration points.

MBP concatemers show improvements up to a factor of four in terms of peak width compared to other calibrants. The calibration range covers the m/z range from 40 kDa (MBP) to 250 kDa (MBP_6) . Optimization of the settings chosen for analysis might also generate nonspecific multimers of the calibration standards and therefore expand the application range at least to 0.5 MDa.

PTh-002
13:30 – 14:40

Relative electrospray response factor in homodimer-monomer equilibrium system of DNA oligonucleotides

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Keywords:
electrospray, non-covalent, DNA oligonucleotides, quantification

Novel aspects:
A new model system based on monomer-dimer equilibrium in DNA oligonucleotides is introduced to directly determine relative electrospray response factor experimentally, which enables accurate binding affinity quantification in non-covalent complexes.

Abstract:
ESI-MS allows one to directly quantify the affinity in non-covalent complexes since all the species present in solution can readily be detected and monitored in mass spectrum. The relative peak intensities of binding partners and complex usually correlate with the abundances of interacting species in solution. However, differences in ionization efficiency, ion transmission, and detection efficiency can disturb the peak ratios and compromise the result of the binding affinity determination. While equal responses can be assumed for non-covalent complexes where a small ligand binds to a macromolecular receptor in a confined deep binding pocket as, for instance, in case of enzyme-substrate, enzyme-inhibitor, and receptor-hormone complexes, different responses are expected in the case of macromolecular oligomeric complexes.
We studied the relative response factors (*R*) in a monomer-dimer equilibrium. DNA oligonucleotides were chosen as a model system for a number of reasons : (*i*) DNA exists in two distinct forms of the single strand (ssDNA) and the double helix (dsDNA) ; (*ii*) the transition between the ssDNA and dsDNA happens in an all-or-none fashion and can be described as a simple monomer-dimer equilibrium ; (*iii*) the factors stabilizing the double helix are very well understood, so that the Gibbs free energy of dimerization can be precisely determined experimentally or reliably predicted for a given nucleotide sequence and solution conditions. From the known Gibbs free energy value the association constant (K_a) can be calculated.
A set of model DNA oligonucleotides with very close chemical composition but different nucleotide sequence was synthesized and analyzed with nano-ESI mass spectrometry using a hybrid quadrupole-time-of-flight instrument (Q-TOF Ultima, Micromass, Manchester, UK) in both positive and negative ion modes. For every sample, a dilution series was prepared in order to cover the concentration range from high-nanomolar to middle-micromolar values. The acquired ESI-mass spectra were baseline-corrected, smoothed and deconvoluted using the maximum entropy algorithm. The monomer-dimer peak ratios were calculated and used to establish titration plots. A double-parametric non-linear regression was derived based on the expression for K_a and the material balance, and used to fit the experimental data. The K_a parameter was fixed at the theoretically predicted value for a given oligonucleotide and the *R* parameter was determined from the fit.
Since the chemical structure and composition of the analyzed DNAs is very close, and a dimer is built of two identical subunits that interact through a large interface (a very typical case for biomolecular oligomeric complexes) , we expected very similar ionization mechanism and no difference in ion transmission or detection efficiency for all the tested samples. The relative response factors *R* should therefore be very similar in all cases. However, the *R* values determined for the positive mode varied substantially, while those for the negative ion mode appeared to be very close to each other and were all below 1, which is consistent with a higher response from the monomer. In the positive ion mode, analytes are charged via protonation, and in case of DNA protonation sites are localized in the dimerization interface. The variation in *R* determined for the positive ion mode data can therefore be attributed to the disturbance of the chemical equilibrium during ionization.

PTh-003 Protein Flexibility is Key to Cisplatin Cross-linking in Calmodulin

11:10 – 12:20

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University of Warwick, Coventry, UK

Keywords:
Cisplatin ; Cross-linking ; Calmodulin ; Flexibility analysis ;

Novel aspects:
Flexibility simulation as a fast and low-cost computational method can be a very useful tool for predicting cross-linking pairs in proteins and facilitating MS data analysis.

Abstract:
Chemical cross-linking in combination with Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) has significant potential for studying protein structures and protein-protein interactions. Previously, cisplatin has been shown to be a protein cross-linking reagent and cross-links multiple methionine (Met) residues in apo-calmodulin (apo-CaM) . However, the inter-residue distances obtained from NMR structures are inconsistent with the measured distance constraints by cross-linking : Met residues lie too far apart to be cross-linked by cisplatin. Here, by combining FTICR MS with a novel computational flexibility analysis, the flexible nature of the CaM structure is found to be key to cisplatin cross-linking in CaM. In both calcium-containing and calcium-free forms of CaM, it is found that the side chains of Met residues can be brought together by flexible motions. The possibility of cisplatin cross-linking calcium-containing CaM is then confirmed by mass spectrometry (MS) data. Therefore, flexibility analysis as a fast and low-cost computational method can be a useful tool for predicting cross-linking pairs in protein cross-linking analysis and facilitating further MS data analysis. Finally, flexibility analysis also indicates that the cross-linking of platinum to pairs of Met residues will effectively close the nonpolar groove and thus will likely interfere with the ability of CaM to bind to protein targets, as was proved by comparing assays for cisplatinmodified/unmodified CaM binding to melittin. Collectively, these results suggest that cisplatin cross-linking of apo-CaM or CaCaM can inhibit the ability of CaM to recognize its target proteins, which may have important implications for understanding the mechanism of tumor resistance to platinum anti-cancer drugs.

PTh-004

13:30 – 14:40

High resolution hydrogen/deuterium exchange mass spectrometry maps contact surfaces of VEGF and a novel recombinant monoclonal antibody fragment (CIGB-166A)

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Keywords:
Hydrogen, Deuterium, Exchange, VEGF, epitope

Novel aspects:
It is the first time that VEGF has been studied by HDX-MS and that this methodology has been applied to determine the epitope of VEGF against a monoclonal antibody fragment

Abstract:
Vascular endothelial growth factor (VEGF) is an endothelial cell-specific angiogenic and vasculogenic mitogen. VEGF also plays a role in pathogenic vascularization which is associated with various clinical disorders, including cancer and rheumatoid arthritis.
A novel recombinant monoclonal antibody (CIGB-166A, Mab) has been designed as a VEGF antagonist, preventing its interaction with its receptors and therefore acting as a potential drug for cancer treatment. The antibody fragment recognizes the VEGF dimer exclusively but its binding site to VEGF is not yet known. Mapping the antigen epitope is a key step in defining antibody specificity, predicting cross-reactivity, in assay development, rational vaccine design, and in understanding the fundamental aspects of protein-protein interactions.
Amide hydrogen/deuterium exchange (HDX) coupled with proteolysis and high resolution MS has become a powerful method to study protein dynamics, protein-ligand interactions, and protein-protein interactions. In general, when performing antigen-antibody experiments by HDX-MS, regions of the antigen with different deuterium content in the presence or absence of the antibody are defined as the epitope.
Here we describe the HDX followed by semi-simultaneous disulfide bond reduction-proteolysis and on-line desalting LC-MS/MS strategy used to determine the epitope of the VEGF-Mab interaction and highlight the challenges to achieve high peptide coverage for good resolution protein structural information.
In VEGF, the monomers are covalently linked by two symmetrical disulfide bonds. Moreover, three more intrachain disulfide linkages and intra and inter hydrogen bonds compose a highly structured dimer. Deuterium labeling of VEGF alone has already protected exchangeable regions corresponding to the contact surfaces of both monomers and those protected by the protein folding itself. Therefore, deuterium labeling of VEGF-Mab implies that regions in VEGF protected by HD exchange are not only those corresponding to the antigen-antibody interaction but also to those regions protected by the complex structure.
In this study, we have designed two complementary labeling strategies to get insight into the VEGF interaction sites with the antibody fragment. Strategy 1 performs the deuterium labeling of VEGF and Mab separately, before inducing their interaction. The complex is then incubated in aqueous buffer, therefore interchanging all deuterium except those in the binding regions and in other protected regions due to the tridimensional structure of the complex. Strategy 2 induces the complex first and than performs deuteration. In this case, non labeled regions would indicate VEGF-Mab sites of interaction and other protected regions, including the surface contact regions between monomers. Deuterated and non-deuterated control experiments of VEGF were also performed in the absence of the antibody. Added to the constrain to use proteases working at low pH and low temperature to limit back-exchange during proteolysis, proteins that contain disulfide bridges present additional difficulties to be digested. Disulfide linkages can render extensive segments of the protein inaccessible to proteolysis, and the available proteolytic segments would be too long to allow localization of the site (s) of H/D exchange. Therefore, the disulfide bonds need to be reduced for effective HDX-MS analysis. The reduction cannot be performed before or during the HDX reaction, as it can change protein conformation. Here we performed a semi simultaneous reduction and digestion after H/D exchange and under quench conditions (0 C, pH 2.5) . On-line desalting was then carried with an in-house double valve system, servicing a trapping and analytical column for sample resolution. All system was maintained at 0 C and column eluent was coupled to an LTQ-FT Ultra mass spectrometer. Data processing was done with HDX Sierra Analytics software. Percentage of deuteration for each region of the protein was calculated by comparing the results of the HDX experiments and putative regions of interaction VEGF-Mab were determined.

PTh-005
11:10 – 12:20

Following amyloid fibril formation by ion mobility spectrometry mass spectrometry: are all pathways the same?

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Keywords:
amyloid fibril, ion mobility, noncovalent

Novel aspects:
This study constitutes a thorough comparison between the structures and properties of protein monomers, oligomers and fibrils associated with three different amyloid diseases, and potential methods of assembly inhibition.

Abstract:
Amyloid fibrils are formed when peptides or proteins self-assemble into filamentous aggregates. Such fibrils are associated with over 25 neurodegenerative and pathological human diseases. Despite extensive studies, the mechanism by which these proteins assemble into highly ordered, cross-beta structures remains elusive, and whether the final fibrils or the intermediate oligomeric species are responsible for toxicity is a key matter for on-going debate. The precursor proteins in each case are dissimilar in terms of their native folds and primary sequences, yet the amyloid fibrils produced share common structural and chemical features, such as a cross-beta organisation of the polypeptide chain and the ability to bind dyes such as thioflavin-T and Congo red. The observation of a common structural architecture for amyloid fibrils has motivated efforts to elucidate the molecular mechanisms of fibril formation *in vitro* with the aim of revealing possible targets for therapeutic intervention.
Electrospray ionisation ion mobility spectrometry mass spectrometry (ESI-IMS-MS) is an ideal technique for monitoring changes in the structure and population of individual biomolecules within heterogeneous samples in real-time. Here, ESI-IMS-MS has been employed to study the folding properties of a range of protein monomers, all of which form disease-related amyloid fibrils *in vivo*, and to monitor the self-assembly of the protein monomer into oligomers over time. For each assembly pathway the mass, stoichiometry, and collision cross-sectional area of all the species detected have been determined. Additionally, the fibril assembly process has been monitored simultaneously using chemical tests and the architecture of the final fibrillar structures investigated use of electron microscopy. Further insights into the structure of the final fibrils have been obtained by the use of limited proteolysis followed by ESI-MS. For each protein, a non-amyloidogenic protein of similar sequence has been used as a control.
A comprehensive comparison between three different amyloidogenic proteins has been made in terms of the conformational flexibility of the protein monomer, the range and dynamics of the oligomers formed *en route* to fibrils, and the structure of the final fibrils. Ataxin-3 is a 42 kDa protein that contains an N-terminal Josephin domain followed by two ubiquitin interacting motifs, a polyQ stretch and a variable C-terminal region. It is the protein responsible for the neurodegenerative polyQ disease Spinocerebellar ataxia type 3, or Machado-Joseph disease. Beta-2-microglobulin is a 99-residue, 7-beta strand, 11.9 kDa protein which is associated with the condition dialysis-related amyloidosis. Islet amyloid polypeptide is a 37-residue peptide hormone produced by beta cells of the pancreas, and is one of the most amyloidogenic peptides known.
The data generated provide insights into three different fibril-producing systems. We have compared the conformational properties of the three different wild-type protein monomers, in addition to the properties of associated protein mutants which have significantly different fibril-forming propensities. The array of oligomers generated in each case, their dynamic nature (assessed by sub-unit exchange), and trends in the relationship between their collision cross-sectional areas with the increasing number of protein sub-units, are all discussed. The structural architectures of the fibrils formed from the three proteins under different conditions *in vitro* have been compared by electron microscopy and limited proteolysis-ESI-MS. Furthermore, a range of small molecules has been used to test amyloid inhibition mechanisms for each protein.

PTh-006
13:30 – 14:40

Structural Characterisation of Protein Complexes by Chemical Cross-linking and Ion Mobility-Mass Spectrometry

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Keywords:
Protein Complex ; Ion Mobility Mass Spectrometry ; Chemical Cross-linking ; Negative Ion Mass Spectrometry

Novel aspects:
Advances in protein complex structure determination by a combination of IM-MS and cross-linking ; Chemical cross-linking analysis in the negative ion mode.

Abstract:
Biological processes are carried out and controlled primarily by a complex, precise network of interacting proteins, which therefore offer potential as novel targets for therapeutic intervention in human diseases. In order to understand and modulate the protein cellular machinery, it is useful to have a molecular understanding of its components and their binding interactions. However, structural characterisation of such intricate protein assemblies often proves difficult due to their heterogeneity and transient nature, and analytical limitations of current structural biology techniques.

Ion-mobility mass-spectrometry (IM-MS) has greatly enhanced our ability to study these systems and extract structural information regarding complex stoichiometry, size and topological arrangement. In addition, chemical cross-linking can be used to discern binding interfaces and provide restraints for molecular modelling applications in which model structures can be generated.

Here we present data demonstrating the effective use of a combination of mass spectrometric approaches to describe low resolution structural models of protein assemblies not amenable to other technologies. We will describe the design, synthesis and application of novel chemical cross-linking reagents for use with negative-ion IM-MS, built around a disulfide bond that undergoes facile cleavage in the negative mode. Such cross-linking methodology in combination with IM-MS collision cross-section measurements can probe the topological arrangement of protein complexes of unknown structure, and we will describe application to systems under study currently in our laboratory including calcium-calmodulin-peptide regulatory complexes, human transcription factor assemblies such as TFIIH, and aggregation products of alpha synuclein.

PTh-007
11:10 – 12:20

The effect of gas molecules for the structural dynamics of GAPDH revealed by mass spectrometry coupled with H/D exchange

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Keywords:
H/D exchange, Gas molecules, Protein dynamics

Novel aspects:
The effect of gas molecules for the structural dynamics of GAPDH revealed by mass spectrometry coupled with H/D exchange

Abstract:
To find new gas-sensing systems, we searches heme-binding proteins in human cell homogenate using heme-modified beads, which decrease non-specific binding. In this experiment, we found that Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) bound with the heme-modified beads having an iron ion. GAPDH, forms homotetrameric complex (MW : 143kDa) , is a member of glycolytic enzymes. The activity is regulated by gas molecules such as Nitric oxide (NO) and hydrogen sulfate (H_2S) , through the post-translational modification, feedback inhibition, and interactions with regulating proteins and ligands. Recently, we discovered a new regulation system for GAPDH by heme and carbon monoxide (CO) . Human GAPDH undergoing such modifications does not only alter its catalytic activity but also triggers entering nucleus to control transcription. Since the relationship between the structural information and the aforementioned functional alterations remains largely unknown, we aimed to determine structural movement of gas-treated enzyme in solution by hydrogen/deuterium (H/D) exchange coupled with pepsin digestion.

GAPDH from *Human* was overexpressed as GST fusion protein in *E. coli*, and purified using affinity chromatography and filtration after protease digestion. H/D exchange was started by mixing GAPDH solutions with D_2O at 22 °C. The pH of the mixtures were 7.1 and the H : D atomic ratio was 1 : 9. GAPDH in D_2O was incubated for 60 min. H/D exchange was quenched by adding acetic acid (pH 2.5) , and digested at 0 °C by pepsin for one minute. The digested GAPDH solution was frozen with liquid nitrogen. Frozen sample was rapidly melted by mixing with 10mg/ml 3,5-dimethoxy- 4-hydroxycinnamic acid in 50 % acetonitrile involving 0.1 % trifluoroacetic acid, and set up on MALDI-TOF MS (AXIMA Performance, Shimadzu) at 10^4 Pa.

Peptides of the pepsin-digested GAPDH were separated in the flight tube, and 34 peaks were identified by MS/MS spectra applying CID and TOF/TOF. We analyzed local protein dynamics of GAPDH using mass shift depending on deuterium incorporation into these peptide, covering 96 % of the whole sequence. To discuss these structural dynamics in three dimensions, H/D exchange map was made by painting each fragment depending on the deuterium incorporation ratio in the X-ray crystal structure. The H/D exchange map clearly showed differences of incorporation ratio into GAPDH among untreated, NO, heme-CO, and H_2S treated. It is known that nitrosylation at Cys152 by NO triggers apoptosis through the entering nucleus to control transcription after interaction with Siah 1 protein. NO-treated GAPDH incorporated more deuterium atoms into interaction region (residues from Val220 to Ala238) with Siah 1. On the other hand, H/D exchange of GAPDH binding with heme-CO showed that the complex forms very rigid structure, which is more than GAPDH-heme complex without CO. Therefore, CO stabilizes GAPDH structure using combination with heme. In this study, we elucidate the structural movement and stability of GAPDH controlled by gas molecules, and discovered a new function of CO.

PTh-008
13:30 – 14:40

A systematic survey of protein kinase specificity by in vitro phosphoproteomic approaches

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Keywords:
phosphorylation, kinase/substrate, proteomics, motif sequence

Novel aspects:
Revealing how protein kinases recognize their substrates using LC-MS/MS-based phosphoproteomics coupled with in vitro kinase assay.

Abstract:
Cellular signal transduction regulates various biological functions. Phosphorylation, caused by protein kinases, is one of the most ubiquitous post-translational modifications, and the kinase/substrate pairs are the main components of cellular signaling network. Recent development of liquid chromatography-tandem mass spectrometry (LC-MS/MS) with effective enrichment methods for phosphopeptides has enabled to identify thousands of phosphorylation sites on substrates. Although all kinases catalyze essentially the same phosphoryl transfer reaction, they are known to display remarkable diversity in their substrate specificity. However, MS-based phosphoproteomics cannot afford to obtain upstream kinase information while it excels at identifying their substrates. This makes difficult to intertwine the kinases with their substrates for revealing the whole picture of signaling network. In this study, we examined how protein kinases recognize their substrates using LC-MS/MS-based phosphoproteomics coupled with *in vitro* kinase assay.

The proteins were extracted from cultured cells, and were subsequently dephosphorylated by alkaline phosphatase to eliminate the intrinsic phosphate group. After dephosphorylated proteins were converted into their substrate peptide forms, the *in vitro* kinase assay was performed using recombinant kinases. After the enzymatic digestions, the control and reacted samples were chemically labeled with stable isotope containing tags individually and were mixed. Phosphorylated peptides were enriched by TiO_2 chromatography followed by LC-MS/MS analysis to identify and quantify peptides phosphorylated by the spiked kinases.

In this experiment, recombinant kinases were reacted *in vitro* with two sets of the substrate pool, which contain the wide range of substrate peptides with the variety of sequence and length. As a first step, we evaluated the effect of the substrate forms on kinase recognition using peptide substrates with full or partial motif sequences. Motif sequence is known to exist around phosphorylated residue and is recognized by their upstream kinases. This examination validates the specificity and affinity of kinases to sequences in substrates. As motif sequences are mainly classified into three groups, which are rich in acid residues (*eg.* Asp and Glu) or basic residues (*eg.* Lys and Arg) motif, and motifs with Pro. Among those motifs, we have generated the basic or acid motif-disrupted peptides using glutamyl endoproteinase Glu-C (protease V 8) , lysyl endoproteinase Lys-C and trypsin. V 8 specifically digests peptides at C-termini of Asp and Glu. For base-rich motifs, Lys-C and trypsin, which specifically cleaves at C-termini of Lys and Lys/Arg, respectively, were selected. Next, for the evaluation of the substrate length effect to kinase recognition, we have made two kinds of peptide substrate pools using the combination of Lys-C and trypsin. The proteins digested by Lys-C are supposed to be longer (cleaved at C-termini of lysine) than the peptides that are doubly digested by both of Lys-C and trypsin (cleaved at C-termini of Lys and Arg) . The increase or decrease of phosphopeptides in respective substrate pool was evaluated by comparing with the result in intact proteins.

Eight kinases including both serine/threonine and tyrosine kinases such as PKA, CaMK, CDK 2 /CycA2, Erk2, CK 1 d, CK 2 a2, EPHB 1 and JAK 2 were performed with fragmented peptide sets and proteins. Consequently, we found that the contribution of the substrate sequence and length to the substrate specificity were highly varied, depending on each kinase. According to the obtained properties of each kinase, we extracted a set of rules governing the substrate specificity. The information will be useful to predict upstream kinases from the properties of phosphorylated peptides identified by LC-MS/MS, leading to unveil signaling network.

PTh-009
11:10 – 12:20

Cellobiose dehydrogenase represents a real challenge for hydrogen/deuterium exchange mass spectrometry

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Keywords:
H/D exchange, FT-ICR mass spectrometry, sample preparation, cellobiose dehydrogenase

Novel aspects:
A special sample treatment including disulfide bond reduction, deglycosylation and optimized digestion strategies is required prior H/D exchange mass spectrometry on cellobiose dehydrogenase protein.

Abstract:
Cellobiose dehydrogenase (CDH), the only currently known extracellular flavocytochrome, is produced by a number of wood-degrading and phytopathogenic fungi. CDH is typically a monomeric glycoprotein consisting of two domains joined by a flexible linker region. The larger catalytic (dehydrogenase) C-terminal domain is FAD-associated while the smaller (cytochrome) N-terminal domain contains a haem. The unique combination of one haem and one flavin within a single protein molecule gives CDH the ability to exchange electrons with both two- and a diversity of one-electron acceptors and thus makes CDH a perspective enzyme for applications in biotechnology and in the field of biosensors and biocatalysis.
To date the crystal structure of full-length flavohaemoprotein has not been solved, because crystallisation of the intact CDH is hampered by the flexible interdomain linker. Therefore, the crystal structures of the flavoprotein and the cytochrome domain were elucidated separately and a model structure of the intact CDH was suggested by molecular modelling and docking studies. However, the exact orientation of the domains in native conformation, possibly being different from the theoretical model, has not been described yet.
Our research strategy is based on the combination of hydrogen/deuterium exchange and mass spectrometry. This technique is routinely used to study protein conformation changes and protein interaction surfaces. However, majority of the proteins appearing in publications are rather easy cases because of their smaller size and absence of heterogeneous modifications. CDH is on the other hand large protein (100 kDa) representing a real challenge due to presence of several disulfide bonds and N-glycosylation. In order to unify glycosylation but retain protein native structure we employed deglycosylation using EndoH. Disulfide bond reduction was achieved by addition of TCEP into the quench solution followed by short incubation on ice. However, this treatment was not offering complete sequence coverage. As we found out, the N-terminal part of the protein (cytochrome domain) requires high concentration of denaturing agents to become digested. But even this treatment is not enough (coverage approx. 70%). Therefore we are now working on other digestion strategies involving slightly elevated digestion temperatures and use of different acid proteases. H/D experiments will start as soon as we find conditions providing complete sequence coverage.

Acknowledgement:
The work was supported by grant P206/12/0503 (GACR) and by Institutional Research Project RVO61388971 (IMIC).

PTh-010
13:30 – 14:40

Improved analysis of biopharmaceutical samples using an MS-only Orbitrap mass spectrometer

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Thermo Fisher Scientific, Bremen, Germany

Keywords:
Orbitrap, Biopharma, Intact Proteins

Novel aspects:
An Orbitrap MS-only instrument was optimized for detection of proteins and a secondary charge detector was added to improve AGC.

Abstract:

Introduction

This work is dedicated to improve capabilities of an MS-only Orbitrap mass spectrometer (second generation Exactive) in analysis of very complex mixtures and biopharmaceutical samples. As intact proteins create fast decaying beat patterns in Fourier-Transform (FT) image current detection systems, the instrument needs to be modified in order to get ability to detect the most abundant signal from the very first beat. When dealing with very complex samples, a dedicated C-Trap charge detection (CTCD) system is shown to improve the accuracy of the prescan-based automated gain control (AGC) . Together with the advanced signal processing, the hardware improvements show a significant improvement for several applications.

Methods

To detect the very first beat within the transient, the insulators within the Orbitrap assembly were modified to allow very fast voltage settling after the ion injection pulse. The design of the entire assembly was made completely symmetrical with respect to stray capacitances. The pre-amplifier circuitry was redesigned to improve recovery time from saturation caused by pulses during injection. In conjunction with advanced signal processing shorter transients can be used to record only the very first beat at high abundance while maintaining high resolving power. To improve AGC, periodic detection of ejected charges by a charge detector was employed. This allows an internal monitoring and correction of the AGC results when necessary.

Preliminary data

Although voltage switching during ion injection into the Orbitrap analyzer disturbs image current detection, the implemented hardware changes reduced the duration of this disturbance from four milliseconds to 250 microseconds. With this improvement, the entire first beat of the image current of intact proteins can be used for data acquisition.

Intact Humira antibody (Abbott Laboratories) was used to show the increase in signal due to detection of the entire first beat of the transient. Shorter transients showed higher signal-to-noise ratio as ion signal is concentrated in first few milliseconds of the transient and remainder of transient adds nothing except noise. This also has an influence on mass calibration of such heavy proteins.

In clinical samples, used for the rapeutical drug monitoring (TDM) , higher molecular species coeluting with the analytes of interest may lead to reduced sensitivity and mass accuracy of the instrument due to wrong estimation of the number of charges in the C-trap by the AGC. It could be shown that with the CTCD system in place, AGC remains effective even under critical conditions so that mass accuracy and correct signal intensity maintain under all conditions.

PTh-011
11:10 – 12:20

Topology of cytochrome P450 2B4 and cytochrome b5 interaction and membrane orientation by photoactivable nanoprobes/chemical cross-linking and mass spectrometry

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Keywords:
photoactivable/chemical cross-linking, membrane protein, HRMS

Novel aspects:
The determination of transient interaction between two membrane proteins using combination of photoactivable/chemical cross-linking and HRMS.

Abstract:
The photoactivable nanoprobe or chemical cross-linking in combination with the high resolution mass spectrometry (HRMS) has been employed to characterize contacts and membrane regions involved in the transient interaction between two membrane proteins, cytochrome P450 2B4 (P450 2B4) and cytochrome b_5. The methionine analog, L-2-amino-5,5-azi-hexanoic acid (pMet), incorporated to the membrane domain of recombinant cytochrome b_5 was used as the photoactivable nanoprobe. The chemical cross-linking by soluble carbodiimide (EDC), a "zero length cross-linking reagent", was employed to form the covalent amide bond between closely located or interacting amino and carboxy-group of amino-acid residues. Although, the photoactivation or chemical cross-linking were accomplished in equimolar catalytic complex of P450 2B4 : cytochrome b_5 reconstituted in DLPC vesicles, SDS-PAGE revealedthe covalent P450 2B4 : cytochrome b_5 hetero-dimers in molar ratio 1 : 1, 2 : 1 and 1 : 2. The excised protein bands from SDS-PAGE were analyzed (after in-gel digestion with trypsin or chymotrypsin) using LC-HRMS (FTICR) mass spectrometry to identify crosslinked amino-acid residues. The protein native structures (at the same conditions of protein reconstitution) were confirmed by observation of the stimulatory effect on enzymatic P450 2B4 *O*-dependentylation activity of 7-pentoxy resorufine in both systems.
Based on our findings with the EDC cross-linking, the determined covalently linked amino-acid residues were located on P450 2B4 proximal surface. On the other hand, using pMet incorporated into membrane domain of cytochrome b_5 revealed amino-acid residues situated on predicted P450 2B4 hydrophobic regions. The computed *in silico* models of P450 2B4 : cytochrome b_5 complex using amino-acids detected in cross-links suggest two different types of cytochrome b_5 orientations : the first allowing potential cytochrome b_5 electron donation to P450, the second one only cytochrome b_5 induced modulation of P450 structural changes. Obtained data are discussed from the aspect of P450 function in organism, the participation in xenobiotics detoxification, drug metabolism and unfortunately carcinogen activation.

The project was supported by Grant Agency of the Czech Republic (P207/12/0627), by Charles University in Prague (UNCE #42) and by Institutional Research Project RVO61388971.

PTh-012
13:30 – 14:40

Structural Examination of hexadecameric and octameric G-quadruplexes, exchange-studies and H/D-exchange on Supramolecular Architectures

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Keywords:
G-quadruplexes, H/D-exchange, Self-assembly ; Ionophores, ESI-FTICR-MS

Novel aspects:
H/D-exchange on supramolecular complexes. Examination of the exchange-behavior between G-quadruplexes.

Abstract:
The synthesis and self-assembling properties of 8-aryl-2'-deoxyguanosine derivatives are described. Our studies suggest that a properly placed acetyl group can increase the stability and specificity of the resulting Guanosine-quadruplex supramolecules by enhancing noncovalent interactions such as hydrogen bonds and π-stacking. The self-assembly of lipophilic dG-analogues could be modulated by replacement of the H8 in the guanine base with a functionalized phenyl group.

The Guanosine-rich sequence of telomeres, ends of chromosomes, have a propensity to form G-quadruplex structures in vitro. In the areas of supramolecular chemistry and nanotechnology, the groups of Davis and Gottarelli have made various lipophilic G-analogues for the construction of self-assembled ionophores, self-assembled liquid crystals, and other molecular devices. The stated importance of Guanosine-quadruplexes makes it appropriate to determine structure function relationships with G-analogues. In particular, G-analogues that are modified at the guanine base could be used to modulate the properties of oligonucleotides or other self-assembled structures containing them.

In our studies we show different buildingblocks of 8-aryl-20-deoxyguanosine
derivatives, which form homoleptic, self-assembled, supramolecular complexes with discrete sizes characterized by ESI-FTICR-MS. With microreactor experiments in front of our ESI-source we were able to estimate the kinetics of exchange between complexes of different buildingblocks in solution.

It also shows that mixtures of hexadecameric and octameric complexes lead to mixed hybrid complexes, which have a dodecameric arrangement. In following MS/MS-studies and theoretical calculations we are also able to draw conclusions about the exchange-mechanism of the buildingsblocks exchange between two complexes.

In ongoing H/D-exchange-studies were perform in gas-phase H/D-exchanges on G-quadruplexes with NH_4^+ and K^+ and subsequent MS/MS-studies to recive a better understanding of the different intrinsec nature of the hydrogenbonds network in the complex.

PTh-013
11:10 – 12:20

Identification of on-pathway intermediates of beta-2 microglobulin fibril formation

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University of Leeds, Leeds, UK

Keywords:
ion-mobility, protein-protein, protein-ligand

Novel aspects:
Use of ESI-IMS-MS to identify species on-pathway to the fibril formation of beta- 2 microglobulin

Abstract:
The ability of amyloidogenic proteins to assemble into large, insoluble fibrillar species is associated with over twenty-five different disease states, such as Alzheimer'sdisease, Parkinson's disease and type II diabetes. In each case, a different protein or peptide self-assembles to form amyloid fibrils, although the final fibril architecture is distinct. The formation of amyloid fibrils *in vitro* can be initiated using a variety of conditions, for example, by denaturation, upon the addition of metal ions or through the introduction of mutations and deletions within the protein sequence. Under each of these conditions, various monomeric protein confirmations and oligomeric precursors exist together in solution in dynamic equilibrium. Identifying which of these species are key precursors to amyloid formation is vital to understand more about the mechanism of formation, an aspect which is essential for the development of amyloid inhibitors to be used as therapeutics, in addition to characterising potentially toxic oligomeric species. However, identifying which states initiate fibril formation can be challenging, as few techniques are able to both separate and characterise such transient species.

Here, the power of ion mobility spectrometry, combined with mass spectrometry, has been harnessed to achieve the separation and characterisation of both monomeric and oligomeric precursors of beta- 2 microglobulin fibril formation. Through comparing species key to fibril formation under a number of different conditions, such as through variation of pH and ionic strength, differences in the pathway of assembly have been identified. Further insights into the importance of these oligomeric species have been gained through analysing the effect of a small-molecule inhibitor of amyloid formation, revealing these oligomeric species are crucial to initiate amyloid formation. In addition, a comparison of the final fibril architecture formed under different conditions has been achieved through the use of limited proteolysis, revealing subtle differences in final fibril structure that are indistinguishable using other techniques.

PTh-014
13:30 – 14:40

Mapping the interaction between Hsp90 and TOM by chemical cross-linking coupled to mass spectrometry

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Keywords:
cross-linking, Hsp90, TOM

Novel aspects:
Chemical cross-linking was used to map the interaction region of Hsp90/TOM complex

Abstract:
The molecular chaperone Hsp90 is a homodimer, where each monomer contains a N-terminal domain (~30kDa) that contains a ATP binding site, followed by an intermediate domain (~30 kDa) and a C-terminal domain (~20 kDa) that is responsible for the dimer formation. The C- and N-terminal domains are also responsible for the interaction with several binding partners. The list of known interacting partners are extensive, however little is known about how these proteins interact with Hsp90.
A recent approach to study protein-protein interactions is the use of chemical cross-linking coupled to mass spectrometry to map the interaction regions of complex subunits. This technique has several experimental advantages, namely high sensitivity, short analysis time and large applicability.
In this work, we described the study of Hsp90/TOM complex by chemical cross-linking and the complex model generated from cross-linking data. The model is compatible with several experimental data already obtained for this complex and reveals how these proteins interact with each other.

PTh-015
11:10 – 12:20

Chemical Cross-linking Coupled to Mass Spectrometry Applied to the Characterization of the Interaction Domains in FimX-PilZ Complex

Mariana Fioramonte[1], Cristiane R Guzzo[2], Shaker C Farah[2], Fabio C Gozzo[1]

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Keywords:
Chemical cross-linking, PilZ, FimX, Structural Proteomics

Novel aspects:
Chemical cross-linking was used to characterize the PilZ-FimX complex.

Abstract:
The PilZ protein was originally identified as necessary for type IV pilus (T4P) biogenesis. Since then, a large and diverse family of bacterial PilZ homology domains have been identified, some of which have been implicated in signaling pathways that control important processes, including motility, virulence and biofilm formation. Furthermore, many PilZ homology domains, though not PilZ itself, have been shown to bind the important bacterial second messenger bis (3'-> 5') cyclic diGMP (c-diGMP) . Some works show that PilZ binds to the EAL domain of FimX, which regulates T4P biogenesis and localization in other bacterial species. These interactions were confirmed in NMR, two-hybrid and far-Western blot assays and are the first interactions observed between any PilZ domain and a target protein.
Chemical cross-linking coupled to mass spectrometry is an attractive tool for structural proteomics when no high resolution method is applicable to the system of interest. In recent years, this approach has been widely used in the study of protein folding, identification of binding partners, monitoring of conformational changes upon ligand binding, characterization of surfaces in protein complexes and probing for solvent residues accessibility. In this work we used chemical cross-linking along with high resolution method (X-Ray crystallography) to map the interaction between FimX and PilZ proteins. The complex crystal was obtained and chemical cross-linking was used to probe the complex structure in solution.

PTh-016
13:30 – 14:40

Inter-molecular interactions of SMC proteins studied by MS under non-denaturing condition and HDX-MS

Susumu Uchiyama[1], Masanori Noda[1], Rie Takino[1], Yuya Miyahara[1], Kazuki Kawahara[2], Yuki Hosokawa[1], Daisuke Motooka[1], Shota Nakamura[1], Tadayasu Ohkubo[1], Kiichi Fukui[1]

[1]Osaka University, [2]Nara Women's University

Keywords:
MS under non-denaturing condition, HDX-MS, chromosome protein, condensation

Novel aspects:
Dimer formation and interaction sites of SMC proteins in solution are firstly identified.

Abstract:
Formation of metaphase chromosomes is essential for proper segregation of genomic information to daughter cells. Pioneering work of Hirano showed that structure maintenance of chromosomes (SMC) proteins play a central role in chromosome condensation. In case of higher eukaryotes, three non-SMC proteins bind to SMC dimer to form condensin complex composed of five subunits. Our proteome analysis of human metaphase chromosomes (Uchiyama, et al., JBC, 2005, Takata et al, 2007 etc.) and RNAi analyses of identified chromosome proteins (Matsunaga et al, Cell Rep., 2012, etc.) showed condensin is one of major proteins in chromosome structure.

Here, we analyzed inter-molecular interactions of hinge domain of human SMC (hSMC-h) by mass spectrometry under non-denaturing condition and hydrogen exchange mass spectrometry (HDX-MS) .

MS under non-denaturing condition showed that hSMC 2 -h forms homo-dimer while most of hSMC- 2 forms hetero-dimer with hSMC- 4 h. These dimer formations were confirmed in solution by analytical centrifugation sedimentation velocity. The homo-dimer formation was disrupted in hSMC 2 -h-GD variant of which three glycine residues were replaced to aspartic acid residues.

We then carried out HDX-MS analyses of hSMC 2 -h homodimer and hSMC 2 -h-GD variant. The results specified three regions which would be responsible for the dimer formation. The regions well reflected the interaction sites for the homo-dimer formation in the x-ray crystal structure of hSMC 2 -h homodimer. The comparison of HDX-MS results between hSMC 2 -h homodimer and hSMC 2 -h/hSMC 4 -h hetero-dimer identified regions which would be involved in the formation of the hetero-dimer.

PTh-017
11:10 – 12:20

StavroX - a software for analyzing crosslinked products in protein interaction studies.

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Martin-Luther-University,Halle,Germany

Keywords:
crosslinking, protein, complexes, analysis, software

Novel aspects:
StavroX is suitable for a wide variety of crosslinking-MS applications. It combines automatic data analysis with an easy-to-use graphical user interface.

Abstract:
Chemical crosslinking in combination with mass spectrometry has matured into an alternative approach to derive low-resolution structural information of proteins and protein complexes. Yet, one of the major drawbacks of this strategy remains the lack of software that is able to handle the large MS datasets that are created after chemical crosslinking and enzymatic digestion of the crosslinking reaction mixtures. Here, we describe a software, termed StavroX, which has been specifically designed for analyzing highly complex crosslinking datasets. The StavroX software was evaluated for three diverse biological systems : (1) the complex between calmodulin and a peptide derived from Munc13, (2) an N-terminal ß-laminin fragment, and (3) the complex between guanylyl cyclase activating protein-2 and a peptide derived from retinal guanylyl cyclase. We show that the StavroX software is advantageous for analyzing crosslinked products due to its easy-to-use graphical user interface and the highly automated analysis of mass spectrometry (MS) and tandem mass spectrometry (MS/MS) data resulting in short times for analysis. StavroX is expected to give a further push to the chemical crosslinking approach as a routine technique for protein interaction studies.

PTh-018
13:30 – 14:40

Electrospray mass spectrometry for detailed mechanistic studies of an aldolreaction catalyzed with L-proline

Mohammed Wasim Alachraf, Wolfgang Schrader

Max Planck Institut for Coal research

Keywords:
Organocatalysis, L-proline, Mechanistic study, Electrospray-ionization.

Novel aspects:
Mechanistic studies of catalyzed aldol reaction

Abstract:
In the last few years, organocatalysis has emerged as a new catalytic methods based on metal-free organic molecules. In many cases, these small compounds give rise to extremely high enantio selectivities. Usually the reactions can be performed under an aerobic atmosphere with wet solvents. The catalysts can be easily synthesized in both enantiomerically pure forms and they are often more stable than enzymes or other bioorganic catalysts. Also, these small organic molecules can be anchored to a solid support and reused more conveniently than organometallic/bioorganic analogues. Herein, we present a mechanistic study of the formation of the aldol condensation product as side product of organocatalytic aldol reaction with L-proline by ESI-MS, and ESI-MS/MS.
The mechanistic studies of the organocatalytic aldol reaction was accomplished by ESI-MS using a Thermo TSQ Quantum Ultra AM triple quadrupole.
Reaction of hexanal was carried out with L-proline as a catalyst (20 % mol) in acetone, which was acting both as solvent and reactant. The reaction was performed at room temperature for 24h. Samples were taken after different time intervals for mass spectrometric study. The reaction was performed at different conditions and deferent techniques were applied (e.g. online monitoring reaction) to study the mechanism of this reaction.
The aldol reaction produce two deifferent products, an addition product and a condensation product, the first one is the desired product of this reaction. The aim of this work is to understand the formation mechanism of the side product and hopefully determine how to avoid production of this side product. There are three potential mechanisms to form this condensation product. The first mechanism is an aldol mechanism, in which the catalyst forms an enamine intermediate with the ketone (here, acetone) . This intermediate reacts in the next step with the aldehyde to yield the second intermediate, which is then hydrolyzed in the last step to form the final condensation product and the regenerated catalyst. The second potential mechanism is a Mannich mechanism, in which the catalyst reacts with the aldehyde to form an iminium cation as a first intermediate. An acetone molecule attacks this intermediate in a nucleophilic attack to produce the second intermediate. In the last step the catalyst splits off to form the final condensation product. The third mechanism consists of the first steps of both the aldol and Mannich mechanisms, where the enamine intermediate of the aldol mechanism reacts with the iminium intermediate of the Mannich mechanism to form the second intermediate. This intermediate has two molecules of catalyst, from which one catalyst splits off and the other hydrolyzed to form the final condensation product. The first step in determining the proper mechanism of such a reaction is to find selective intermediates that can be used as reaction markers for each mechanism. Reaction markers of two pathways were discovered. Although the empirical formula and the mass of the intermediates of both mechanisms is identical, it is possible to determinate the intermediates structure by using ESI-MS/MS according to McLafferty rearrangement fragmentation. Using different techniques, like deuterium exchange in the retro-reaction we were able to exclude one of the two remaining pathways to ensure that only one pathway controls this condensation reaction.
The final results will be presented.

PTh-019 LC/MS and surface ionization techniques in classification of red wine

11:10 – 12:20

Petr Bednar[1], Barbora Papouskova[1], Lucie Hartmanova[1], Karel Hron[2], Jan Stavek[3], Josef Balik[3], Renata Myjavcova[1], Petr Bartak[1], Eva Tomankova[3], Karel Lemr[1]

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Keywords:
LC/MS, Anthocyanin, Variety, MALDI, nanoDESI

Novel aspects:
LC/MS profiling of anthocyanins combined with AAEs and PCA gives robust data for variety classification. MALDI and nanoDESI allow fast wine evaluation directly from intensity of signals of particular anthocyanins.

Abstract:
Profiling of flavonoids and products of their transformation is effective tool for evalutation of authenticity and quality of food products. This task, however, can hardly by done without a reliable and robust method providing long-time consistent results. Combination of (ultra) high performance liquid chromatography with high resolution tandem mass spectrometry undoubtedly represents such a method. Recently we have shown on a complete set of certified red wine varieties that LC/MS profiling of anthocyanins in combination with evaluation of activity of enzymes involved in anthocyanin biosynthesis (AAEs) [1] and advanced statistical data treatment (clr transformation, PCA) allows efficient classification of red wine varieties in northernmost European viticultural area. To our knowledge the study is the first evidence of data structure stability related to anthocyanin profile over long-time (evaluation of four vintages ; analysis of each in respective year) and comprehensive wine sample set in given region [2] . Main features and relations observed during the study can be summarized as follows. Higher content of peonidin derivatives (a higher activity of related methyltransferase) refers to a potential occurrence of teinturiers (i.e. varieties Alibernet, Neronet and Rubinet) when included in the set of analyzed wine samples. It was found out that variety Blaufrankish has low content of acetylderivatives of anthocyanins (lower activity of acetyltransferase) and this fact can be utilized for resolution of this variety. Two-dimenzional display of data (PCA in the from of biplot) emphasizes differences among individual teinturiers, Blaufrankish and partially separate the group of Blaufrankish, Pinot Noir, Blauer Portugieser and Domina varieties. Moreover, suitable division of biplots in a direction of a principal component based on enzymatic activities further allowed a more detailed classification of Merlot and Cabernet Moravia varieties and led to an unambiguous differentiation of variety Saint Laurent from Blaufrankish which has indispensable meaning from the viewpoint of their high occurrence on the Czech market. The above mentioned approach is based on precise quantification of particular anthocyanins from LC/MS data. Surprisingly, a similar degree of classification based on evaluation of AAEs and PCA was allowed one year later simply by direct evaluation of signals of anthocyanins in MALDI spectra of particular varieties. Beside, desorption nanoelectrospray (nanoDESI) allowed revelation of addition of a teinturier (variety Rubinet) into a common red wine for the purpose of its artificial color enhancement [3] . Those results indicate a high potential of surface ionization techniques for wine authenticity evaluation and the approach will be also discussed in this communication.

Acknowledgement
Authors thank to Grant Agency of Czech Republic (project P206/10/0625) . This work was supported also by the projects MSM 6198959214 and Operational Program Research and Development for Innovations European Social Fund (project CZ.1.05/2.1.00/03.0058) of the Ministry of Education, Youth and Sports of the Czech Republic.

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PTh-020
13:30 – 14:40

Higher-Order Structural Characterization of an IgG2 Monoclonal Antibody by Disulfide Mapping and Hydrogen Deuterium Exchange Mass Spectrometry

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Keywords:
Antibody, IgG2, Disulfide bond, HDX

Novel aspects:
An analytical workflow combining proteolytic mapping and HDX MS analyses definitively characterized several batches of an IgG 2 mAb.

Abstract:

Introduction:
Recombinant human monoclonal antibodies (mAbs) are widely used in biotherapeutics research and development as potential therapies for various diseases. After antibody production several analytical techniques focused on primary and higher-order structural analyses are conducted to adequately characterize these complex therapeutics. Recent reports detailing the characterization of IgG 2 mAbs revealed the presence of disulfide-linked structural isoforms, termed IgG 2 -A, IgG 2 -B, and IgG 2 -A/B. In this study, we present an analytical workflow to automatically map out the disulfide -linked isomers using data independent LC/MS acquisition and disulfide bond mapping informatics tools. Once the peptide map information is obtained, hydrogen deuterium exchange mass spectrometry (HDX MS) using on-line pepsin digestion and peptic peptide mapping was used to investigate the consistency of multiple IgG 2 batches.

Method:
A recombinant human IgG 2 mAb was produced in several batches at Pfizer (Chesterfield, MO) . Each IgG 2 batch was digested with trypsin without reduction. The digest was separated using UPLC directed into a Q-Tof mass spectrometer. The LC-MS data were processed in software that assigns disulfide bonds based on peptide theoretical masses and high collision energy fragment ions. For HDX MS experiments, each IgG 2 batch was diluted into deuterated solutions, quenched at certain times and injected onto a pepsin column for online digestion. The peptic peptides were separated by UPLC into a Q-Tof mass spectrometer to measure the deuterium incorporation. The amount of deuterium in several peptic peptides was compared using differential plots produced by automated HDX data processing software.

Preliminary Data:
Informatics tools developed at Waters confirmed the identification of the disulfide-linked structural isoforms in the IgG 2 mAb. Several interchain and intrachain linked peptides were automatically identified from the LC/MS^E data. This workflow improves the speed and consistency of identifying predicted and non-predicted disulfide linked peptides in mAbs.

The HDX MS experiments, which combined online pepsin digestion and automated peptide assignment, afforded high sequence coverage of both light and heavy chains (88% and 93% respectively) . The proteolytic digestion with pepsin produced consistent peptide cleavages over each labeling time-course and IgG 2 lot. The automated software assigned deuterium uptake levels to glycopeptides containing G 0 F, G 1 F, and G 2 F N-linked glycans, the most common post-translational modifications in mAbs. We observed interesting deuterium exchange kinetics for a peptide representing the C-terminus of the light chain (VTHQGLSSPVTKSFNRGE***C***, residue numbers 196 to 214) . The major IgG 2 isoforms present in this mAb are the IgG 2 -B and IgG 2 -A/B. Each of these isoforms contain an interchain disulfide bond between the light chain (at position 214) to the heavy chain hinge region. The deuterium exchange profile of this particular light chain peptide exhibited EX 1 -like behavior, as observed as having multiple deuterium distributions at long deuterium exchange times. We attribute this behavior to the presence of multiple disulfide isoforms present in the mAb. Furthermore, the same exact deuterium uptake profile was also observed for this peptide in the other batch of IgG2. HDX MS combined with disulfide bond mapping was found to be a powerful approach to assess the primary and higher-order structure IgG 2 mAbs across multiple production batches.

PTh-021
11:10 – 12:20

Compound search technique for overlapping peaks of LC/MS with a unique algorithm for simultaneous multicomponent identification

Yuka Noritake[1], Haruo Shimada[1], Yoshimasa Nakatani[1], Rakan Matsui[1], Kazumasa Kinoshita[2], Satoru Kamikubo[2], Yasuo Shida[3]

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Keywords:
search algorithm, multicomponent identification, rapid analysis, DART-MS, LC/MS

Novel aspects:
The key to efficient compound search is how to analyze overlapping spectra. We showed the simultaneous multicomponent identification of complex mass spectra from cream and emulsion using the unique algorithm.

Abstract:
Introduction: Compound analysis is important for companies producing products such as cosmetics, medicine, and food through research and development, product development and quality control management. Under the current industrial situations, the rapid and simple analysis techniques have been required to increase their efficiency and cost performance.
In general, liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry are widely used methods for the analysis of complex mixture. Although these methods are beneficial for compound analysis, they have two bottlenecks from the view point of analytical efficiency. One is a time consuming process such as sample preparation and development of LC conditions for separation. Another is a difficulty in data analysis such as peak separation because of limited capacity of conventional algorithms in search software for compound identification. For example, they can identify completely separated peaks by focusing on the extraction of a single mass spectrum derived from one component in a mixture. On the other hand, it is a time consuming process to analyze data that have peaks consisted of two components with the same retention time and an apparently single peak that is actually overlaps of multiple compounds. For the shortcut to data analysis, currently we have developed the unique algorithm to search mass spectra of mixtures simultaneously, which enables us to identify multiple components easily and exclude the bottlenecks as described above. In this study, we showed rapid identification of components in complex spectra obtained from MS analyses of the cream and emulsion with the algorithm. The result indicated that it could be versatile with a direct analysis in real time (DART) and an infusion analysis of LC/MS for the rapid compound analysis. Our poster focuses on a new compound search technique with the unique algorithm for the compound analysis of complex materials (pharmaceutical cream and emulsion) and its application data with the visualization of peak information.

Materials and Methods: Cream and emulsion samples containing active ingredients (lidocain, diphenhydramine, tocopherol acetate) and cream base (methyl benzoate, ethyl benzoate, etc.) were dissolved in acetonitrile. The identification of the compounds was carried out by a quadruple time-of-flight mass spectrometer (Bruker MicrOTOF-Q 2) equipped with HPLC system (Shimadzu prominence XR) with an electrospray ionization (ESI) interface or a DART interface. Separations were performed on a reversed-phase C_{18} column at 40 degrees C. The mobile phase was a simple gradient of acetonitrile-water. For DART analysis, the sample was applied on the glass rod, which was followed by exposure to metastable helium gas. The data obtained from the measurements of LC-ESI-MS and DART-MS were successively assigned to the respective standard mass spectra by using the algorithm.

Results: The active ingredients in the cream and emulsion were eluted successively within ten minutes by LC/MS. Components appeared in the mass spectra were assigned using the algorithm for simultaneous multicomponent identification. The peaks of lidocain and diphenhydramine were identified as a single component, respectively. The peak of tocopherol acetate was assigned with overlapping components of cream base. In spite of the simultaneous elution of many components with the infusion analysis of ESI-MS, each component was identified and corresponded to each standard spectrum. The components in the spectrum obtained from DART-MS were also identified.

Conclusion: In conclusion, the algorithm used in this study enabled simultaneous search of mass spectrum of mixture without peak extraction from complex peaks. The components in cream were not only identified without any pre-examination of HPLC for the peak separation, but also identified without any separation process in the case of DART and an infusion analysis of LC/MS. Our study indicated that this algorithm could be useful for the rapid identification of multicomponent.

PTh-022
13:30 – 14:40

To determine the activity of Essential Oil from Blumea eriantha from Indian origin by HSGC and GCMS.

Jitendra G Kelkar[1], Deepti Kulkarni[1], Ajit Datar[1], Dheeraj Handique[1], Aarti Karkhanis[1], Prajakta Pednekar[2]

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Keywords:
Essential Oil phyto-constituents by HSGCMS

Novel aspects:
Blumea eriantha has shown excellent anti microbial activities. Its components have been identified by HSGC and GCMS. The absorption of Essential oil by plasma shows selectivity.

Abstract:

Introduction

Aromatherapy is a healing art whose roots are laying in antiquity. It is a form of alternative medicine that uses volatile plant materials, known as essential oils, and other aromatic compounds for the purpose of altering a person's mind, mood, cognitive function or health. The essential oils have been used therapeutically for centuries ; there is a little published research on many of them. A significant body of research on pharmaceutical and therapeutic potentials of essential oils is therefore necessary.

In the present work we have selected the following plant of Indian origin to study various healing potentials of its essential oil :

Blumea eriantha* Family: *Compositae; Asteraceae.

This plant is selected as detailed systematic work on therapeutic properties of essential oils has not been reported in the literature.

In the current research work the antibacterial potential of the essential oil, specifically against skin pathogens are evaluated. The antioxidant potential and the safety of the essential oil are also evaluated by cytotoxicity and dermal toxicity study. In this article, we are discussing the analysis and identification of ingredients present in Essential oil from the above plant using Head Space GC and GCMS.

The plant material was collected from Seawoods, around Mumbai which grow in plenty between the months of October to March.

Method

The Essential oil was extracted from the plant by hydro distillation using Clevenger Apparatus. The extraction conditions like temperature, weight of plant material, rate of distillation were optimized so as to get maximum yield of essential oil.

The chemical properties were analyzed using following techniques :

The method was developed on Gas Chromatography (GC) , Shimadzu GC2010 with FID equipped with Tekmar Teledyne HT 3 Head Space Accessory.

The GC method then was transferred to Shimadzu, GCMS (QP 2010 Ultra) for identification of various phyto-constituents of essential oil using EIMS library (NIST) and Terpenoids Library using relative retention indices.

Absorption of Essential oil by Plasma was studied by using the raw rabbit plasma spiked with known amount of essential oil and the absorption of the components of essential oil was studied by using above Head Space GC and GCMS System.

The Microbial Activity of Essential oil was studied on skin pathogens and cancer cells.

Preliminary Data

Shimadzu GCMS model QP2010 Ultra with Tekmar HT 3 Head Space Analyzer played a great role in identification of components of essential oil from plasma. The phyto-chemicals were identified. The absorption of Essential Oil Analysis in plasma has revealed the selective absorption of some of the components. Further work on the selectivity and active ingredient in the oil responsible for the activity is being done.

The microbial activity has been carried out and the oil has shown very good activity on the skin pathogens namely : Propioni bacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes and it showed activity against all of these at low concentration levels.

It has also shown activity against the cancer cells, namely : ACHN (Renal Cancer) ,Panc 1 (Pancreatic cancer) , Calu 1 (lung cancer) , H460 (Non small cell lung cancer) , HCT116 (Colon cancer) .

PTh-023
11:10 – 12:20

Screening of antioxidants present in unripe Manilkara zapota fruit of Indian origin by using LCMS/MS.

Shailendra A Rane[1], Deepti Bhandarkar[1], Rashi Kochhar[1], Shruti Raju[1], Bhairavi Saraf[1], Ajit Datar[1], Jeetendra Kelkar[1], Bhagyashree Jadhav[2]

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Keywords:
Antioxidant screening by using LCMS/MS

Novel aspects:
LCMS/MS can be used for screening of antioxidants present in unripe Manilkara zapota fruits, for their potential application as new source of natural antioxidants for food, nutraceutical and pharmaceutical industries.

Abstract:
Introduction
Antioxidants are the compounds that act as radical scavengers, prevent the radical chain reactions of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the process of lipid peroxidation.
Antioxidants found in fruits and vegetables play an important role via their protective effects against the onset of aging-related chronic diseases. The objectives of this study is to screen the antioxidants present in unripe *Manilkara zapota* fruit, which is commonly available in India, and to indicate that it can become a new source of natural antioxidants for food, nutraceutical and pharmaceutical industries. The LCMS/MS is used to identify the presence of antioxidants from the plant.
Method
Antioxidants were extracted from unripe *Manilkara zapota* fruit followed by purification using Solid Phase Extraction (SPE) . Eluate from SPE was subjected to Low Volume Evaporator (LVE) with final reconstitution in methanol.
The extract was then analysed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera system coupled with LCMS-8030 triple quadrupole system of Shimadzu Corporation. The use of LCMS-8030 ultra high speed mass spectrometer, which is compatible with UHPLC system, aided in keeping the ultra fast and high sensitivity without any deterioration in the quality of data. The use of Nexera with High Speed LCMS/MS has facilitated not only in achieving fast method development but also in getting high sensitivity in MS/MS mode.
Preliminary Data
Antioxidants were extracted from unripe *Manilkara zapota* fruit and UHPLC method was developed for the analysis of the same. The extract was also subjected to mass spectrometric analysis using triple quadrupole system.
Polyphenol antioxidants like catechin, epicatechin were observed in the extract. Their presence was confirmed by comparing m/z values obtained with those cited in the literature. Product Ion Scan spectra for selected precursor ions gave further confirmation of their presence.

PTh-024 Rapid development of functional extensions for mass spectrometry using freeware Mass++

13:30 – 14:40

Howell E Parry[1], Satoshi Tanaka[1], Tsuyoshi Tabata[2], Ken Aoshima[2], Yoshiya Oda[2], Shinichi Utsunomiya[1], Shigeki Kajihara[1], Koichi Tanaka[1]

[1]Shimadzu Corporation, [2]Eisai Co., Ltd.

Keywords:
freeware, plug-in, proteomics, metabolomics, multi-vendor

Novel aspects:
Mass++'s unique plug-in architecture makes it easy for third parties to extend the extensive core feature set with original functionality which may be distributed freely.

Abstract:

Introduction

Mass++ was originally funded by CREST (Core Research for Evolutional Science and Technology) and developed by Eisai. Mass++ 2.0.0 development was taken over by Shimadzu Corporation with continued support from Eisai, and funding from FIRST (Funding Program for World-Leading Innovative R&D on Science and Technology).

Mass++ is freeware for mass spectrometry analysis that can be downloaded from **http://www.first-ms3d.jp/**. Mass++'s primary objectives are :

1. To provide essential functionality for proteomics and metabolomics analysis.
2. To support a wide range of vendors' data file formats.
3. To be easily extendible using plug-in technology.

Mass++'s rich feature set, implemented as plug-ins to the core software, includes chromatogram, spectrum, heatmap and 3D viewers, data pre-processing, advanced peak detection and quantitative analysis.

Support for a wide range of data file formats has been implemented - Shimadzu (LCMSsolution, GCMSsolution), Applied Biosystems (Analyst, AnalystQS), Thermo Fisher (Xcalibur), Waters (MassLynx), mzXML/mzML and MSB (original format).

Mass++ is distributed as freeware. Mass++'s unique plug-in architecture makes it easy to use Mass++ as a base platform onto which original functionality may be implemented as plug-ins.

Method

Mass++ has a "plug-in type" concept ; by handling plug-in types, a plug-in can respond to Mass++ events (for example, when a spectrum is displayed, a DRAW_SPEC event occurs and any plug-in could respond by annotating additional information onto the graph) or it can define itself as a certain type of plug-in, and it will appear as a new option in a dropdown list within Mass++. The types of plug-in that can be created include :

- Data file input
- File export
- Peak detection (spectrum, chromatogram, 2D)
- Charge detection
- Database search
- Peak filter
- Peak analysis
- RT (retention time) alignment

To support third party developers who want to write original plug-ins, Mass++ ships with :

- Comprehensive documentation
- Microsoft Visual Studio development wizard.

This presentation will show, step-by-step, how easily new functionality can be developed with the Mass++ platform, using as an example a simple plug-in to eliminate unwanted detected peaks based on a user-specified height threshold.

The steps for creating a new plug-in are as follows :

1. Create a plug-in project using the development wizard
2. Define the user interface and handler functions etc. in definition XML files
3. Implement handler functions
4. Copy the built plug-in DLL and XML files into the Mass++ plug-ins folder

Result

Newly-created plug-ins are auto-detected the next time Mass++ is run, and their functionality automatically appears in Mass++, for immediate use. It is hoped that third party developers will be encouraged to take advantage of Mass++'s rich core functionality via its SDK to develop their own original plug-ins. New plug-ins may be distributed freely.

References

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PTh-025
11:10 – 12:20

UHPLC-MS/MS, an Alternative Solution to Conventional Biosensor Approach for Quorum Sensing Signalling Molecules Detection in Complex Environmental Samples

Chuan Hao (Grant) Tan[2,4], Kai Shyang Koh[4], Scott A Rice[2,4,5], Yan Zhou[2,3], Staffan Kjelleberg[4,5], Wun Jern Ng*[2,3], Peiting Zeng[1], Zhaoqi Zhan[1]

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Keywords:
Quorum Sensing, Biosensor, Quantification, UHPLC-MS/MS

Novel aspects:
Identification and quantification of thirteen AHLs in a complex activated sludge system were demonstrated for the first time using UHPLC-MS/MS

Abstract:
*Corresponding author

Introduction

Quorum sensing (QS) signalling is critical for coordinating the social behaviours of bacteria, i.e., regulating biofilm development [1]. Acyl-homoserine lactones (AHLs) consist of a homoserine lactone ring and an acyl side chain, at variable lengths, oxidation states and saturation levels, are the most common signals employed by the bacteria. As AHLs are highly susceptible to elevated temperatures, alkaline conditions, and are often degraded rapidly by other microbes living within the same niche, it is always a real challenge to characterize and quantify AHLs in the natural environments [2]. Conventionally, AHLs are detected by biosensor-dependent assays, which are time-consuming and with limited sensitivity detecting all range of AHLs [3]. Here, a UHPLC-MS/MS method is developed to overcome the limitations of the biosensor-based AHL detection.

Experimental

Naturally occurring AHLs, from the activated sludge of a lab-scale bioreactor system, were extracted by dichloromethane. The samples extracted were analysed on UHPLC-MS/MS tandem quadrupole system (Shimadzu LCMS-8030) using a Shim-pack XR-ODS column (2 x 100 mm, 2.2µm). A total of thirteen synthetic AHLs, ranging from C4 to C14, with various oxidation states, were used as reference AHLs for the analysis. Automatic MRM optimization was applied to each standard to determine the MRM transitions for subsequent sample analysis. A pair of MRM transitions was selected for each standard. The MRM transition that exhibited higher intensity was used for quantification analysis, while the other for confirmation of the AHL identity. In addition, MS full scan coupled with synchronised survey scan was employed to identify possible existence of other AHL structures with predicted m/z values.

Results and discussion

A rapid and sensitive quantitative MRM method for the AHLs was established using UHPLC-MS/MS. This UHPLC-MS/MS based method allows for detection and identification of AHL identities with high level of confidence, which would not be achievable otherwise by the conventional biosensor-dependent assays that generally require the AHL samples to be first separated on a thin layer chromatography (TLC) plate, followed by the detection with a specific bacteria biosensor overlay. Using the synthetic AHLs as standards, in the blank sample matrix, good repeatability of the UHPLC-MS/MS method was demonstrated. Despite the presence of complex matrix components, which are highly inhibitory to the ionization of the AHL molecules with ESI interface, the limit of detection (LOD) for all the thirteen AHL standards were found to be approximately 0.1 ppb to 1.0 ppb. These LOD values are comparable to various biosensor-based AHL detection methods ranging from 0.05 ppb to 1000 ppb. However, unlike the biosensor assays, which are often bias towards specific types of AHLs with different acyl side chain lengths and oxidation states, the UHPLC-MS/MS method is unequivocally sensitive to all the AHLs studied at various oxidation stages. This was demonstrated by the detection of naturally occurring AHLs in activated sludge samples, with acyl side chains ranging from C 4 to C10-HSL and with or without carbonyl-substitution at low concentrations. On the other hand, the analysis using *Agrobacterium tumefaciens* A136 biosensor was only able to detect the presence of carbonyl-substituted C 8 -HSL (C 8 -OXO-HSL) in the same samples extracted. Moreover, an additional MS full scan coupled with synchronized survey scan approach allowed further detection of other AHL structures beyond the thirteen synthetic AHL standards that were present in the samples, which would be otherwise not detectable by the biosensor methods.

Conclusions

It was clearly demonstrated that UHPLC-MS/MS as a rapid, sensitive and high-throughput alternative to the conventional biosensor-dependent approach in validation and quantification of quorum sensing signalling molecules in the complex environmental samples.

PTh-027 Strategy for key odor component discovery using GC×GC-TOFMS

11:10 – 12:20

Fumihiko Tsuchiya, Fumie Kabashima, Wataru Uchikawa, Michiko Kanai

LECO Japan, Tokyo, Japan

Keywords:
GCxGC, TOFMS, Fisher Ratio, Flavor, Foods

Novel aspects:
The workflow by non-targeted analysis using GCxGC TOFMS is an efficient method to extract potential candidate compounds from the huge quantities of peak data.

Abstract:

Introduction

The investigation of aroma compounds has been growing in importance in several analytical fields such as food science and the environment, as well as clinical medicine. In such samples, there are many kinds of flavor compounds which contribute to the overall sample flavor profile in a synergistic way. Therefore, comprehensive profiling of the aroma components is essential. GCxGC TOFMS allows non-target analysis and comprehensive fragrance screening due to the enhancements in selectivity and sensitivity provided by two dimensional GC (GCxGC) and by Time of Flight mass spectrometry (TOFMS) . In this presentation we show an example of the comparison of the fragrance components of Awamori, specialty distilled liquor in Okinawa, that has been stored in three different types of vessel, stainless steel, glass and pottery bottles, for twenty years. There are significant differences in flavor between samples due to the long aging process and the method of storage. We applied a non-target analytical method using GCxGC TOFMS to characterize each sample and determined typical compounds defining sample characteristics. We present a workflow for this study and discuss the efficiency of the method for flavor/fragrance analysis.

Method and Materials

One micro liter of each Awamori sample was dispensed into a tall vial (10 mL) and diluted with 1 mL of distilled water. After capping of the vials, aroma components were collected using SPME (DVB/CAR/PDMS) at 60° C, for twenty minutes. The GCxGC data were collected on a Pegasus® 4 D GCxGC-TOFMS system (LECO Corporation) and processed using ChromaTOF® Version 4.41 software. We repeated the measurements four times for each sample to allow a variance analysis. All peak lists tables for the samples were subjected to a peak alignment step in the software to generate a spread sheet which could be exported into the multivariate analysis software JMP08.

Results

More than three thousands peaks were detected for each sample. In order to compare samples we summarized all peak lists into a spreadsheet using the peak alignment function in ChromaTOF 4.41 software. Additionally we calculated Fisher Ratios for each compound in the list. The Fisher Ratio which is a variance ratio between class-to-class and within-class variance is a powerful tool to eliminate peaks that do not show significant difference among the data sets. We sorted the Fisher Ratio of each compound in the peak list in descending order. Since the higher Fisher Ratio is generated by good repeatability and significant differences, the top 150 compounds were assumed to be important candidates as that contribute to the characteristics of the sample. The list of candidates includes many aromatic compounds (e.g. esters, terpenes, sulfur compounds, etc.) . The compound name and peak area data in the list can be used to express the relative abundance ratio of each compound with a 100% stacked bar graph. This workflow provides not only information about compound identity but also quantitative data. The candidate list can also be subjected to multivariate analysis, and showed definite differences between samples in a principal component analysis. The result indicates that this workflow by non-targeted analysis using GCxGC TOFMS is an efficient method to extract potential candidate compounds from the huge quantities of peak data.

PTh-028
13:30 – 14:40

Coordination Polymer Formation Observed by Cold-Spray Ionization Mass Spectrometry

Kazuaki Ohara, Kentaro Yamaguchi

Tokushima Bunri University, Sanuki, Japan

Keywords:
CSI-MS, coordination polymer, crystal, halogen interaction

Novel aspects:
Coordination species, which extends to form coordination polymer, was detected by cold-spray ionization mass spectrometry.

Abstract:
Coordination bonding is one of the most attractive interactions for the construction of nanometer-sized molecules such as catenane, rotaxane which is dumbbell shaped molecule, and guest encapsulating compound. The interaction provides the reaction profiles as well as selective molecular recognition capability. Analytical techniques such as NMR spectroscopy, X-ray analysis and other spectroscopic approach have been developed with a progress of macromolecular synthesis and applied to reveal coordination structure or selective molecular recognition. However, conventional mass spectrometry (MS) has not been adopted for these labile complexes even by using soft ionization, such as electrospray ionization (ESI) . Cold-spray ionization (CSI) at low temperature has made it possible to observe labile coordination species without any dissociation. CSI-MS has been applied to unstable compounds, Grignard reagents, host-guest molecules, multiply stranded DNAs, and other unstable biological compounds.

Metal-organic porous materials mainly categorized as metal-organic frameworks (MOFs) and coordination polymers (CPs) are generally obtained by hydrothermal synthesis and the diffusion method, respectively. After the material is synthesized, structure of the product, generally obtained as the solid, is determined by powder or single-crystal X-ray structure analysis.

However, the coordination species which is soluble and grown up to be insoluble coordination polymer has not received any attention for a long time. In recent research, the crystal growth of MOFs was investigated by time-resolved in situ X-ray scattering. Although this analytical method yields a part of the metal-organic structure determined by the combination of the ligand and the metal before crystallization, it is not sufficient to discuss the detailed structures of the coordination species in solution. In our previous study, it was succeeded to observe organic network structure with hydrogen bonding of steroid compounds over 10-mer in solution by CSI-MS and confirms its network structure accommodated into crystal. The molecule having weak interaction each other in solution generally exhibits good agreement with the structure observed in crystal.

In this work, we observed the coordination behavior in solution by CSI-MS comparing to the crystalline coordination structure. To discuss coordination, Cu (I) iodide was selected as a metal which potentially had two competitive weak interactions : coordination bonding and metal-halogen interaction. Although Cu (I) iodide was a widely used catalyst for the 1,3-dipole cycloaddition reaction to obtain multiply substituted 1,2,3-triazole, the tetrahedral CuI easily gave 1-D chain cluster with metal-halogen interaction. Complexation of organic ligand having multiple nitrogens with the copper potentially gives several coordination compounds. In addition, the two competitive weak interactions furnish highly complicated pathways for the construction of coordination polymers. We investigated a soluble coordination species in CSI-MS and discuss coordination structure in solution compared with coordination polymer.

PTh-029
11:10 – 12:20

Mass Spectral Libraries for Analysis of Complex Plant Metabolomics Datasets - Algorithms

Wm Gary Mallard[1], Steve Stein[1], Yuri Mirokhin[1], Nirina Rabe Andriamaharavo[1], John Halket[2]

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Keywords:
AMDIS, plant metabolomic libraries, deconvolution,

Novel aspects:
A library of plant metabolites is developed of recurrent unidentified spectra. Specific algorithms to improve the data quality for complex data files are discussed.

Abstract:
A principal problem in plant metabolomics is the large number of unidentified components in the samples. However, these complex mixtures may be characterized by frequently recurring components in terms of the spectral and retention parameters of the components. We have developed a series of methods for the analysis that provides high quality spectra with well characterized retention parameters for recurrent compounds that can be used in search libraries. Here we discuss the algorithms that are used to filter and sort these data. The example data used here is a set of essential oil samples of Vetiver taken from multiple commercial sources and analyzed at multiple dilutions.

The GC/MS data files are deconvoluted using the NIST deconvolution software AMDIS and a target library composed of spectra from the NIST MS library (NIST11) as well as spectra from the literature for components in Vetiver. The analysis was limited to a region of the chromatogram between 16 and 31 min on DB-5. Typically 120 to 200 components were extracted by AMDIS with about one half identified using the target library. All unidentified components were then analyzed to determine which recurred. AMDIS was run in a mode that rejected data if there were too few ion peaks that had similar time histories (model peaks) , if the S/N of the spectra was so low that the minimum ion peak that could be extracted from the noise was not at least 0.5% of the base peak. The resulting set of unidentified peaks provided between 200 and 400 unknowns that were recurring. These unknowns were then searched against the entire NIST database and where identification was made (initially about 5 % of the unidentified components) , the new compounds were placed in the target library.

The spectra from the recurring unknowns were further processed using a set of algorithms that examined the distribution of peaks in the spectrum. These algorithms were designed to find spectra that a human expert would find "unlikely ". The preliminary version of the algorithm focused on the appearance of clusters of peaks. Thus spectra with numerous isolated peaks with no apparent isotope peaks were rejected. In addition, spectra with very few peaks were rejected as were spectra that appeared to have unreasonable neutral losses. Preliminary application of these criteria rejected almost 80 % of the unidentified spectra. The resulting 20% were incorporated into a target database and then rerun against the data files to confirm the quality of the data. The resulting data sets were examined exactly as was done with the initial data set and the same set of filters were applied to define any remaining high quality spectra that could be added to the library.

The challenge in analyzing these complex mixtures is that there are two common problems that give poor spectra - strong overlap between components so that it is not clear that there are multiple components (resulting in extra peaks in a component) and small components that are partially coeluting with larger components containing common ions that make the deconvolution of the these ions difficult (resulting in missing peaks in the component) . Specific algorithmic tools were developed to identify cases where one or the other of these problems was occurring and spectra where these problems could be identified were rejected. The success of these algorithms was tested on the Vetiver data prior to their being applied to a far larger set (over 1000 data files) of essential oils.

The library of these recurrent, high quality spectra is available for download.

PTh-030

13:30 – 14:40

Structure estimation of triacylglycerol by comparing calculated peak pattern with high energy CID spectrum using MALDI-TOF-TOF tandem MS

Takaya Satoh, Kubo Ayumi, Hizume Takuhiro, Sato Takayoshi, Ueda Yoshihisa

JEOL Ltd.

Keywords:

Structure estimation, MALDI-TOF-TOF, lipid, triacylglycerol

Novel aspects:

Structure estimation of triacylglycerol by comparing calculated peak pattern with high energy CID spectrum using MALDI-TOF-TOF tandem MS was performed

Abstract:

Introduction :

Lipids are the molecules consisted of backbone structure, such as glycerol and, which combined with one or more fatty acids combined with backbone. Characters of lipids vary according to structure of fatty acids in those. Structural analysis of lipids have been performed using tandem mass spectrometer. Especially tandem mass spectrometers which are capable of high energy CID have been well performed for obtaining detail structure of fatty acid moieties, such as position of double bond and branching, by observing signal yielded by charge remote fragmentation. However, product ion spectra of lipids which have two or three fatty acids observed by those instruments is quite complex to interpret structural information, because signals derived from each fatty acids are overlapped in a product ion spectrum. Therefore, simple interpretation methodology of product ion spectra is necessary. In this study, automatic interpretation based on database search was performed. There are some databases of product ion spectra of lipids, but those which has enough product ion spectra of lipids by high energy CID is not found. And, development of new database required the much time, effort, and many money to acquire product ion spectra of lipids standards. So, we attempted to develop the estimation method of product ion spectrum of lipid based on the empirical rule. This empirical rule was established by comparing the product ion spectra between the several kinds of lipids that have a similar structure each other.

We report the estimation method of high energy CID product ion spectra of lipids and the validation result of the method by employing triacylglycerol as a test sample.

Method :

A JMS-S3000 "SpiralTOF " MALDI-TOF-TOFtandem MS (JEOL Ltd., Tokyo, Japan) was employed for obtaining product ion spectra of several kinds of TAG whose chemical structure have already known. The product ion spectrum of one of TAGs in low or middle mass region were estimated by consulting past literature [1]. And the product ion spectra in high mass region were estimated based on the observed product ion spectra of TAG that has similar structure. The product ion spectra of several kinds of TAG (54 : 3) were estimated by using above method, and those were stored into the database. MassBank search system were used for product ion search between observed product ion spectra and estimated spectra.

Result :

The product ion spectra of several kinds of isomer of TAGs (54 : 3) were estimated and stored on to the private database of MassBank. The observed product ion spectrum of TAG (16 : 0,18 : 1,18 : 2) were applied for MassBank search by using the private database. As a result of database search, correct candidate was clearly distinguished from some structural isomers of TAG (54: 3). On the other hand, identification of positional isomers was not clearly performed (MassBank search gave almost same score for each isomers) because product ion spectra in high mass region were exact same that was induced by CRF of fatty acid moieties. But, characteristic peaks which enable identification of positional isomers were observed. If the search algorithm can be improved for TAGs by using such characteristic peaks, product ion spectrum search system based on MassBank has possibly to be useful tool for lipids identification.

[1]. Pittenauer,E. ; Allmaier, G. ; *J. Am. Soc. Mass Spectrom.* **2009**, 20, 1037-1047.

PTh-031
11:10 – 12:20

Identification of antioxidants in Fructus aurantii and its quality evaluation3 using a new on-line combination of analytical techniques

ZONGTAO LIN[2], JING DONG[1], HONG WANG[2], YING XU[2], YUKI HASHI[3], SHIZHONG CHEN[2]

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Keywords:
Fructus Aurantii, On-line derivatisation, Flavonoids, Antioxidant identification, HPLC-DAD-ESI-MS-PCD-LPFCL

Novel aspects:
An on-line system developed here can be applied for rapid, accurate identification of multiple active constituents in Fructus Aurantii

Abstract:
A new on-line method for simultaneous identification and monitoring of antioxidants in *Fructus aurantii* was established by coupling high performance liquid chromatography-diode array detector-electrospray ionisation-ion trap-time of flight-mass spectrometry with post-column derivatisation and luminol-potassium ferricyanide chemiluminescence (HPLC-DAD-ESI-IT-TOF-MS-PCD-LPFCL) . While the HPLC fingerprint, structural identification and radical scavenging profile were rapidly obtained by an on-line assay using ultraviolet (UV) absorption, MS and LPFCL, details of the precise substitution patterns of various structures were achieved through UV absorption using PCD addition of shift reagents. Twenty-five flavonoids were identified by either their PCD and MS data or comparison with reference substances. Data collected both from chromatograms and activity profiles of 12 samples revealed significant differences among samples from different habitats. The results showed that this method was rapid and precise, and therefore would be an effective and sensitive method for biocompounds analysis and quality evaluation for complex food and medicinal samples.

PTh-032
13:30 – 14:40

Use specific proteins of Taiwan tea as biomarkers for the certification of origin

Han-Ju Chien, Chien-Chen Lai

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Keywords:
Oolong tea / 2 D-PAGE / MS / biomarker

Novel aspects:
To our knowledge, this is the first report of using the proteomic strategy to distinguish Taiwan teas from the foreign teas.

Abstract:
Oolong is a traditional Chinese tea *(Camellia sinensis)*, one of the major teas in Taiwan, processing with partially fermentation, the major components are tea polyphenols and caffeine. Many studies have proofed that drinking of tea appropriately can prevent cardiovascular disease, obesity and cancer. Recently, the cheap cost and the poor-quality of the foreign tea have made an enormous impact on Taiwan teas. The blend of low-quality foreign teas with Taiwan teas resulted in the low competitiveness of Taiwan teas exportation. Furthermore, consumers are worried about the unclear place of production and indistinct safety of import foreign teas. In this study, we focus on finding the key biomarkers to differentiate between import foreign teas and Taiwan teas. The sources of Taiwan oolong teas come from Nantou, Chiayi, Taitung, New Taipei City, Taichung, Ilan, Hualien, Yushan, Lishan, Alishan, Deer Valley, Lushan, Dong Ding Mountain, and ones of foreign oolong teas come from Thailand, Indonesia, and South Vietnam. Until now, several studies have showed that the proteome expression profiles of plant leaves altered with the variant environment, including harvest time, composition of the soil, insect damage, disease, cultivation, and the pollution of pesticide and heavy metals, it have been carried out on different kinds of stress in plants and biomarker discovery. Herein, 2 D-PAGE (two-dimensional polyacrylamide gel electrophoresis) was applied to the comparative proteomics analysis of the teas with different production place. These specific proteins could be used to distinguish Taiwan teas from the foreign teas.

PTh-033
11:10 – 12:20

JobRequest - an easy-to-use software platform for proteomic analysis - and ProteoAnalysis, its application for protein identification.

Tsuyoshi Tabata[1], Akiyasu C Yoshizawa[2], Takayuki Kimura[1], Tatsuji Nakamura[1], Ken Aoshima[1], Yoshiya Oda[1], Shigeki Kajihara[2], Koichi Tanaka[2]

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Keywords:
Platform Proteomics Identification JobRequest ProteoAnalysis

Novel aspects:
We developed an easy-to-use and scalable software platform for proteomic analysis, and its application for protein identification. They will be publicly released on the web.

Abstract:

Introduction

For proteomic analysis, it is often necessary to modify the analysis procedure for the large amount of data acquired. However, it takes time and effort to rewrite the "controlpart " of the entire process, such as the control part of the execution sequence for multiple programs, or the administration for multiple computational servers, etc. Moreover, this rewrite is not related to the proteomic analysis itself. For the UNIX environment, some tools for these purposes are already available, although utilizing them presents a significant hurdle for experimental scientists to overcome, because they require an advanced knowledge of computer systems. Hence, an *easy-to-use* software platform for the bioinformatics analyses of proteomic data is widely anticipated.

Implementation of *JobRequest*

We developed the "JobRequest " analysis platform in order to overcome the situation mentioned above : this program has a scalable architecture that works on one or more PCs, and controls the hardware and the entire process on both Windows and UNIX compatible (Linux) environments via a graphical user interface. JobRequest itself can be run on both Windows and Linux, accompanying the Apache web server. This platform automatically executes system-related tasks and scalability-related tasks such as designing parameter setting screens, scheduling tasks and assigning low load PCs to each analysis process : hence users are relieved from the burden of implementing these functions, and can focus on writing the actual analysis programs. For the analysis programs in JobRequest, web-standard languages, such as PHP, Ruby, and Perl, are supported. We will introduce the basic functions of JobRequest in the poster presentation session.

Implementation of *ProteoAnalysis*

As a practical application of JobRequest, we also constructed the "ProteoAnalysis " software suite on the JobRequest platform for the identification of proteins from raw mass spectrometry data. By inputting multiple raw data, this suite automatically executes several processes as follows, in parallel :

(1) Making peak lists using **Mass++** [1] functionality.
(2) Identification of proteins from MS/MS spectra using X! Tandem as the search engine and searching using **MSPTM-DB**, which we have developed [2] ,is also available.
(3) Filtering results by search scores.
(4) Adding detailed annotations such as Gene Symbols, Gene Ontology terms, literature information etc.

Each result is automatically merged and integrated into a single file and displayed on the screen or saved in a comma separated value (CSV) file which can be opened in Excel. Both JobRequest and ProteoAnalysis will be freely available to download in the immediate future. ProteoAnalysis will also be published as a web service.

References

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[2] Yoshizawa, A.C., et al., MSPTM-DB：a known PTM database for high-speed and accurate search available on the "ProteoAnalysis" web site. *submitted to this IMSC*.

PTh-034
13:30 – 14:40

Fragmentation analysis of glucuronide metabolites using hybrid ion-trap/orbitrap mass spectrometer with electrospray ionization

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[1]Astellas Pharma Inc, Tsukuba, Japan, [2]Yokohama City University, Yokohama, Japan

Keywords:
Fragmentation, Glucuronide, CID, ESI, ISD

Novel aspects:
An MS approach for determining the site of glucuronidation in glucuronide metabolites was found by using CID, pos/neg ion mode, and ESI-ISD methods.

Abstract:
[Introduction]
Identifying metabolic pathways of a drug candidate is important not only for its pharmacokinetic characterization, but also for the estimation of its safety and efficacy. The chemical structures of metabolites are mainly estimated based on mass fragmentation data in drug discovery. It is known that glucuronidation is one of major non-CYP metabolic pathways. However the metabolic site of glucuronidation is difficult to determine using mass fragmentation data, because the glucuronide is easily decomposed during electrospray ionization (ESI) process and rapidly dissociated during fragmentation process. In this study, the in-source decay (ISD) in ESI and collision-induced dissociation (CID) of glucuronide metabolites were investigated using eight model compounds.

[Method]
Acyl-glucuronides of probenecid, ketoprofen, naproxen, and telmisartan, and β-glucuronide isomers of raloxifene and ezetimibe were used as model compounds. Experiments were performed using a LTQ Orbitrap Velos hybrid mass spectrometer with electrospray ionization interface (Thermo Fisher Scientific Inc., San Jose, CA) . The solution of glucuronide standards at a concentration of 20 μmol/L, delivered by a syringe pump at a flow rate of 5 μL/min, was mixed through a T-piece with an HPLC effluent. The mobile-phase flow rate was set at 0.2 mL/min. The heated capillary was set at 330°C, and the spray voltage was kept at 4.5 kV (positive ion mode) or 3 kV (negative ion mode) . The sheath gas flow (N_2) and auxiliary gas flow (N_2) were set to 50 and 10 arbitrary pressure units, respectively. Capillary and lens voltages were systematically optimized for the protonated or deprotonated molecule using autotune. The CID was used to acquire the product ion spectra using helium as a collision gas in the ion trap.

[Results and discussion]
The ionization efficiency of each deprotonated molecule $[M-H]^-$ was little different among eight model compounds in negative ion mode. However that of protonated molecule $[M+H]^+$ was so much different in the positive ion mode. In the case of the compounds ketoprofen and naproxen, the protonated molecule was not detectable at any conditions. This may be due to low-efficiency of protonation since they have no nitrogen atoms in their chemical structures. The extent of the deconjugation in ESI-ISD was very low in the negative ion mode except for a compound probenecid, On the other hand, the glucuronide of the compounds ezetimibe, probenecid and telmisatan was dissociated easily in positive ion mode. The extent of the deconjugation of telmisartan glucuronide was higher under acidic conditions than neutral conditions. The results obtained about the telmisartan in the positive ion MS data and chemical conditions suggested that the dissociation or deconjugation takes place through the attack of proton to the analyte molecules. The fragmentations in the CID spectra of six model compounds showed that the glucuronide moiety was rapidly dissociated even when lowest collision energy was applied, while the CID spectrum of two ezetimibe glucuronides did not show a rapid dissociation. When the compounds have a beta-lactam ring like the ezetimibe, a rapid dissociation of the lactam moiety occurred. Here we obtained the information about the MS and chemical conditions for the dissociation or deconjugation of glucuronide metabolites, and using the information obtained here the glucuronidation sites could be obtained on the basis of the fragmentation analysis.

PTh-035
11:10 – 12:20

Proteomic analysis of salt-responsive ubiquitin-related proteins in rice root.

Yen-Fu Chen[1], Chang-Sheng Wang[2], Chien-Chen Lai[1]

[1]Institute of Molecular Biology College of Life Science, National Chung Hsing University, Taichung, Taiwan, [2]Department of Agronomy, National Chung Hsing University, Taichung, Taiwan

Keywords:
Rice, salt stress, ubiquitination, LC-MS/MS

Novel aspects:
These salt-responsive ubiquitinated proteins may be helpful in further elucidating the molecular mechanisms involved in protecting plants from salt stress.

Abstract:
Ubiquitination of proteins plays an important role in regulating a myriad of physiological functions. In plants, ubiquitination governs such processes as xylogenesis, senescence, cell cycle control, and stress response. However, only a limited number of proteins in plants have been identified as being ubiquitinated in response to salt stress. Rice (Oryza sativa) seedlings exposed to salt stress, and the proteins of roots were extracted then analyzed using Western blotting against ubiquitin. Differentially expressed ubiquitinated proteins were identified by LC-MS/MS and quantified by the Exponentially Modified Protein Abundance Index. In addition, we performed a gel-based shotgun proteomic analysis to detect the total ubiquitinated proteome that may be involved in response to salt stress. We found ubiquitinated proteins that were expressed in response to salinity such as pyruvate phosphate dikinase 1, heat shock protein 81-1, probable aldehyde oxidase 3, plasma membrane ATPase, and cellulose synthase A catalytic subunit 4 [UDP-forming] . Most of those ubiquitinated proteins were found to be involved in metabolism and transport functions. The result demonstrates that the differentially expressed ubiquitinated proteins with different rice species in salt stress by using proteomics technology and mass spectrometry.

PTh-036
13:30 – 14:40

Application of high speed LC-MSMS technology towards unambiguous characterization of degraded products of Lenalidomide, an anticancer molecule

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Keywords:
High Speed LC-MSMS Technology

Novel aspects:
The detailed charaterization of base degraded impurities of Lenalidomide has been accomplished for the first time, the structure of the impurities is elucidated unambiguously.

Abstract:
Lenalidomide RS (3-(4-amino-1-oxo1, 3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione) is a potent immunomodulatory drug with antineoplastic, antiangiogenic and anti-inflammatory properties. It is also used in combination with dexamethasone for the treatment of multiple myeloma. It is being evaluated in combination with other agents for the treatment of other type of tumors, including solid one. One of the requirements of drug substance as well as the drug product is their stability under harsh environments such as temperature, humidity, light and oxidizing agents. It is also important that the drug substance / drug product has to be evaluated at wider pH range to understand the degradation of the product. Following ICH guidelines, degradation studies are carried out by acid hydrolysis, base hydrolysis, UV irradiation studies and peroxide degradation. Knowing the potency of the lenalidomide, many attempts have been made to understand the stability of the compound simulating different environmental conditions towards separation and identification of degradation products using HPLC and LC-MS methods. Though the molecular weight of some of the degraded products has been identified, no attempts have been made to fully characterize the degraded products to understand the mechanism of degradation of lenalidomide. The high speed LC-MSMS technology is applied, for the first time, to unambiguously characterize the forced degraded products by appropriately proposing the MS fragmentation pathways for the degraded products.
Lenalidomide was in-house synthesized following a four step synthetic process. Briefly, esterification of 2-methyl-3-nitrobenzoic acid in the presence of thionyl chloride in methanol provided the methyl ester of 2-methyl-3-nitrobenzoic acid. Benzylic bromination of this methyl ester using N- Bromosuccinimide provided the bromo compound, isolated bromo compound was coupled with 3-aminopiperidine-2, 6-dione hydrochloride using triethylamine in the mixture of acetonitrile and dimethylformamide gave 3-(4-nitro-1-oxoisoindoline-2-yl) piperidine-2,6-dione. Hydrogenation of 3-(4-nitro-1-oxoisoindoline-2-yl) piperidine-2,6-dione using palladium on carbon in dimethylformamide provided crude lenalidomide, crude was purified using methanol to obtain a pure lenalidomide. The purity of the lenalidomide was found to be 99.96% (% area) by HPLC.
The degradation was simulated under (a) acid hydrolysis, (b) base hydrolysis, (c) peroxide oxidation and (d) photo degradation under sunlight. It was observed that the degradation was the highest and instantaneous when base hydrolysis simulation was carried out, whereas acid hydrolysis did not show any degradation products in HPLC analysis. The purity of lenalidomide got reduced from 99.96% to about 97.0% under peroxide oxidation and exposure to sunlight. The number of degraded products formed under these conditions were 7 to 8 components of intensity varying from 0.02% to 1.6%. However, in this presentation the characterization of degraded products due to base hydrolysis is discussed in detail.
Though the instantaneous formation of degradation products of lenalidomide by base hydrolysis is known, the application of high speed scanning capability of Shimadzu LCMS-8030 has enabled us to characterize these products. We observe two major impurities as degradation products having the relative retention time (RRT) of 0.47 and 0.67 minutes respectively. It is interesting to observe that the molecular weight of both the degradation products is found to be 277 Daltons. The negative MSMS analysis of both the components has shown that the structures are different with unique fragmentation pattern. The product at RRT 0.47 is found to be 4-(4-amino-1-oxoisoindolin-2-yl) -4-carbamoylbutanoic acid and the product at RRT 0.67 is found to be 2-(4-amino-1-oxoisoindolin-2-yl) -4-carbamoylbutanoic acid. The structures are assigned unambiguously by exploiting the high speed scanning rate of LCMS8030 under MSMS environment.

PTh-037
11:10 – 12:20

Metabolite ID analysis using UNIFI, a novel database driven software platform approach for screening and understanding metabolism

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Keywords:
Metabolite ID, LC/MS, Informatics,

Novel aspects:
Metabolite identification for processing and sharing of high resolution mass spectrometry datasets using a scientific library controlled LCMS software package

Abstract:
Current software platforms have been limited generally to single experiment analysis types, although these approaches have and continue to serve us well, the majority were designed for linear experimental designs and answers. As individual assay performance in Met ID and DMPK departments have continued to improve with LCMS platform advances in recent years, researchers are focusing on ways to increase value from their complex datasets. The approach defined is a Met ID software system that uses the latest generation PC hardware (64bit, multi-core capabilities) , latest generation hardware (High resolution UPLC/MS Quant of platforms) and software (scientific library data management) to more accurately screen, confidently interrogate datasets and report the data to the user in a highly customizable format.

Samples were analyzed on G 2 and G 2 -S Quant of MS platforms equipped with I-class AcquityUPLC systems. Metabolite analysis of several compounds was performed using a prototype version of UNIFI 1.6 software. Software contains a scientific library which is used to enter and retrieve information about the compounds being analyzed and is able to interface. Samples were automatically processed to provide a comprehensive list of metabolites which can then be filtered or interrogated to suit both the analytical and reporting needs of the user. Integrated and flexible mass accuracy filtering, decimal mass filtering, isotopic recognition and fragment analysis along with metabolite relationship mapping will be discussed.

Dataset processing using UNIFI was compared and contrasted to current generation software product, Metabolynx XS version 2.0. Data was analyzed using latest Apex peak picking algorithms with advanced multicore processing capabilities. Processing takes advantage of builtin scientific library functionality which manages both compound and metabolite identification specific criteria and allows users to define and generate metabolite relationships within a single LCMS software package. Current methodology relies heavily on linear single compound, single dataset, single answer approaches. With a scientific library underpinning, users are able to both build and retrieve metabolite relationship information across datasets and samples that were historically difficult to manage as a single report. Stronger integration of in silico tools, metabolite prediction, isotopic pattern analysis and more flexible mass defect filtering will be discussed. Will demonstrate how drug metabolism departments will have the ability to manage instrument and server assets remotely across the network. The network based infrastructure also facilities scientifically sharing and disseminating data and knowledge across labs and across networks. DMPK datasets are processed for both generating qualitative structural and relationship pathways as well as for quantitative analysis and putting the datasets into meaningful context within a single, integrated processing and reporting environment.

PTh-038 Metabolite Identification Workflow Software

13:30 – 14:40

Graham A McGibbon[1], Vitaly Lashin[2], Alexey Aminov[2], Dmitry Mitushev[2], Hans de Bie[1]

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Keywords:
software, metabolite identification, biotransformation database

Novel aspects:
Association of structures from metabolism data including fragmentation and involvement of software to manage full biotransformation schema linked to interpreted spectra.

Abstract:

Background

Metabolite structure determinations made by review of LC/MS extracted chromatograms and spectra are vital but time-consuming. Gains in sensitivity, acquisition rates and resolution of mass spectrometry instruments offer more rather than simpler data, so the identification process must increasingly involve software for data, structure and information management to satisfy mounting pressure for workplace efficiency. However, software absolutely needs to handle chemical structures and results documentation as well as facilitating the processes of mass spectrometry data interpretation to truly be effective and reliable. While elemental composition assessments are improving, finding metabolic 'soft spots' of new chemical entities and summarizing biotransformation pathways for drug candidates remain key activities in pharmaceutical discovery and development. This work describes a new software approach facilitating overall structure assignment workflows including biotransformation knowledge management for metabolite identification.

Methods

New ACD/Labs software was investigated for processing mass spectrometry data acquired from any major instrument system. Workflows for LC/MS data from several vendor instruments, including different quadrupole time-of-flight analyzers, were examined. Parent structures were provided and metabolites were identified by automated feature finding and subsequent determination of mass and from accurate mass systems also elemental composition. The software made comparisons of parent and metabolites to further localize sites of modification when possible. The software was able to conveniently handle structurally ambiguous metabolites using Markush representations. The structures were stored together with extracted mass spectra, including assigned fragment ions, and could be reported or exported to a biotransformation database. Various manual searches of the saved structures and data were then conducted to demonstrate that feasibility.

Preliminary Results

LC/MS data files from metabolite identification studies were processed post-acquisition. Along with the data files from various samples, the appropriate parent structure was imported and translated to a new software format capable of managing the various channels of LC/MS, LC/MS/MS, LC/MSn data and associating chemical structures with those data. With parent structures provided, metabolites were identified by automated feature finding then their masses determined and, if mass accuracy permitted, elemental compositions. The software made comparisons of parent and metabolites to further localize sites of modification when possible. The software was able to conveniently handle structurally ambiguous metabolites using Markush representations. Files storing structures together with extracted mass spectra, including assigned fragment ions, could be saved or reported. Also, a major aspect for workflow is that any data could also be exported along with the structure assignments to another software module designed for biotransformation knowledge management. The latter accommodates entire metabolic pathways, with searchable spectra and structures. Various manual searches of the structures, substructures and data saved in a biotransformation database were then conducted to demonstrate that feasibility.

PTh-039
11:10 – 12:20

Integration of spectral library in standard data processing pipeline for shotgun proteomics data

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Keywords:
Pre-search Spectral library, Shotgun proteomics

Novel aspects:
The integration of a spectral library, used as a pre-search, in standard data processing pipeline for shotgun proteomics data.

Abstract:

Introduction

Over the past years the usage of spectral library search tools was established in shotgun proteomics. Instead of identifying the same peptide over and over again by a sequence database search engine like SEQUEST, spectral library search uses a library of spectra identified with high accuracy. The restriction to previously measured spectra avoids a lot of computational overhead. Search tools like SpectraST, MSPepSearch or BiblioSpec are freely available and a lot of work was spent on building accurate and confident libraries, which is the crucial part of the identification procedure. Here we present the integration of a spectral library search engine (SpectraST) into an automated pipeline for shotgun proteomics data analysis (Proteome Discoverer) .

Methods

The usability of spectral library search as fast and sensitive first level search to speed up the peptide identification in a standard shotgun proteomics data analysis workflow will be presented.
All samples were measured on a Thermo Scientific LTQ Orbitrap Elite instrument coupled to a Thermo Fisher Easy nano-LC. The data was analyzed using a pre-release version of Thermo Scientific Proteome Discoverer. All spectral libraries used were from NIST. For data analysis different workflows were used. Search results of spectral library searches were compared to classical database search engines like SEQUEST and Mascot. The benefit of the usage of spectral library search as pre search to a standard database search is investigated in a sequential Proteome discoverer workflow.

Abstract

A HeLa and a *E.Coli* sample were processed using the different workflows. Comparing spectral library search to classical database search shows a decrease in search time. The number of spectra identified in the spectral library search is smaller compared to the database search due to the limitations of the used libraries to previously identified high confident peptides, but almost all identifications from spectral library search have a corresponding highly confident identification in the database search. Using a spectral library search as an identification method for a subset of spectra prior to a database search is still considerably faster than a method based on identifying peptide sequences by database search alone.

PTh-040
13:30 – 14:40

Application of MALDI-TOF Mass Spectrometry Characterization in the Development of Nano-porous Metallophthalocyanin Sensor Electrodes

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Keywords:
Metallo 4', 4 ", 4' ", 4 "" tetraamine Pthalocyanines, nitric oxide (NO) detection, Nanoporous Sensors

Novel aspects:
Application of MALDI-TOF mass spectrometry as a conclusive characterization tool for Metal Tetranitro- and Metal Tetraamino-Pthalocyanins

Abstract:
Metallo 4', 4 ", 4' ", 4 "" tetraamine Pthalocyanines (MTAPcs) have attracted attention for the preparation of chemically modified electrodes for electrocatalytic reactions and sensor applications and they are typically immobilized onto the electrode surface as a stable polymeric film by electropolymerization that proceeds through the oxidation of the -NH 2 substituent. In this work we report the successful synthesis of MTAPc monomers (using a 2-step synthetic protocol) via reduction of the corresponding Tetra-nitro Phthalocyanine (MTNPc) intermediates using oil bath or microwave heating methods for M=Cu, Mn, Zn, Fe and Pt. The synthesis of Metal tetranitropthalocyanins (MTNPcs) involves the cyclotetramerization of pre-functionalized phthalonitrile, phthalic acid and phthalic anhydride precursors together with an appropriate metallic salt, with subsequent reduction to give the desired amino monomers (MTAPcs) .

Apart from the use of UV-Vis and 1 H-NMR spectroscopies and elemental analysis, the products of synthesis were also extensively characterized using MALDI-TOF mass spectrometry. In the analysis of experimental MALDI-TOF mass spectrometric data of MTNPcs and MTAPcs, attention was paid to compare our results with simulation results of expected products ("IsotopeViewer version 1.0 " was used to provide the simulation of the [M] +· isotopic clusters) . MALDI-TOF mass spectrometry has been found to be an exceptionally useful characterization tool in providing conclusive evidence of success of the synthesis.

In general, MALDI-TOF mass spectra of the MTNPc and MTAPc complexes provided intense signals due to the molecular radical ion ([M] +·) . The mass spectra of MnTNPc, PtTNPc and ZnTNPc complexes show minor fragmentations due to photodeoxygenation of the peripheral nitro substituents. The mass spectra of FeTNPc and FeTAPc show the [M] +· of the metal complex as well as those of the metal-free H 2 TNPc and H 2 TAPc compounds, strongly suggesting the demetallation of FeTNPc and FeTAPc under the intense laser pulses. For PtTNPc, the [M] +· species (calculated : 887.05489, found : 886.9318) is 23 % in intensity relative to the base peak of 903.9324 (100%) . The base peak is deduced to be due to the [M+OH] + species, where M is the molecular weight of PtTNPc.

The second part of our work involves studies on the electrochemical detection of dissolved nitric oxide (NO) in phosphate buffered saline (pH 7.4) by electropolymerized-Metallo 4', 4'', 4''', 4'''' tetra-amine Phthalocyanine (poly-MTAPc) modified electrodes. For fabrication of electrodes for electrocatalytic reactions and sensor applications, MTAPcs are typically immobilized onto the electrode surface as a stable polymeric film by electropolymerization that proceeds through the oxidation of the -NH 2 substituent. The electrocatalytic poly-MTAPc coating is in the form of arrays of nanotubes and fibers - this gives rise to improved sensitivity and detection limit through the provision of more electroactive area.

Two modified electrode systems (the flat glassy carbon electrode (GCE) and a Pt-coated nanoporous anodic aluminium oxide (AAO) membrane) were selected as the bare substrates for poly-MTAPc modification. Our Differential Pulse Voltammetry and Amperometry (DPV and DPA) results showed that of the two, the high-density poly-MTAPc nanotube array within the modified AAO electrode provided a high faradaic (signal) to charging (background) current ratio leading to 10-15 times improvement in sensitivity and a 10 times drop in detection limit.

PTh-041
11:10 – 12:20

Highly accurate chemical formula prediction tool utilizing high-resolution mass spectra, MS/MS fragmentation, heuristic rules, and isotope pattern matching

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Keywords:
chemical formula, heuristics, isotope pattern, MS/MS, prediction

Novel aspects:
The problem of chemical formula elucidation has been addressed previously, but this is the first comprehensive and user-friendly implementation in an open source mass spectrometry profilingtool (MZmine 2).

Abstract:
Mass spectrometry is commonly applied to qualitatively and quantitatively profile small molecules, such as peptides, metabolites, or lipids. Modern mass spectrometers provide accurate measurements of mass-to-charge ratios of ions, with errors as low as 1 ppm. Even such high mass accuracy, however, is not sufficient to determine the unique chemical formula of each ion, and additional algorithms are necessary. Mass spectrometry vendors provide their own software modules for chemical formula prediction, the capabilities of which vary by vendor. We developed a universal software tool for predicting chemical formulas from high-resolution mass spectrometry data. The tool is based on the use of a combination of heuristic techniques, including MS/MS fragmentation analysis and isotope pattern matching. The performance of the tool was evaluated using a real metabolomic dataset obtained with the Orbitrap MS detector. The true formula was correctly determined as the highest-ranking candidate for 79% of the tested compounds. For compounds smaller than 250 Da, the true formula was always predicted correctly and, in 67% of the cases, as the only candidate conforming to the heuristic rules. On average, the heuristic algorithms reduced the search space for potential formula candidates 40-fold. The novel isotope pattern-scoring algorithm outperformed a previously published method in 64% of the tested Orbitrap spectra. Great emphasis was put on user-friendly operation of the tool, which is now freely available as part of the open-source MZmine 2 framework and its source code can be accessed within the MZmine 2 source code repository.

Reference : Pluskal T., Uehara T., Yanagida M., Highly Accurate Chemical Formula Prediction Tool Utilizing High-Resolution Mass Spectra, MS/MS Fragmentation, Heuristic Rules, and Isotope Pattern Matching. *Anal Chem* (2012) , **84** (10) : 4396-403

PTh-042
13:30 – 14:40

SBSE probe desorption GC-IT-MS analysis of degradation products of esfenvalerate obtained by chemical oxidation process

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Keywords:
SBSE-GC-IT-MS ; esfenvalerate ; chemical oxidative degradation

Novel aspects:
Study of degradation of esfenvalerate by using a chemical oxidative process. Utilization of SBSE-GC-IT-MS for extraction of degradation products of esfenvalerate and their identification.

Abstract:
A growing number of investigations have been reported recently showing the widespread occurrence of agrotoxics in the environment, notably in the aquatic compartment. The treatment of these pollutants by oxidative process using hydroxyl radicals (•OH) have been highlighted because of its high efficiency in the degradation of numerous organic compounds and low operating cost. To evaluate the efficiency of this process and monitoring of intermediates and final products, hyphenated chromatographic techniques are indispensable. However, due to the low concentration of pollutants and their degradation products in aqueous medium associated to the incompatibility of the aqueous matrix with conventional GC-MS techniques, some steps of preparation and pre-concentration of the samples are necessary. The SBSE (stir bar sorption extraction) technique combined with hyphenated chromatographic techniques such as GC-MS resulted in rapid analysis, low solvent consumption, higher analytical precision and sensitivity, and has been successfully employed in the extraction of agrotoxics in water at the concentration range of sub-ng L^{-1}. However, SBSE-GC-MS analysis requires thermic desorption of the analytes, and nowadays only one commercial SBSE thermal desorption system is available. On the other hand, an approach called "SBSE probe desorption " is being investigated by us as an alternative to integrate the advantages of SBSE and the power of GC-IT-MS (gas chromatography-ion trap-mass spectrometry) as the detection technique. In this procedure, the SBSE bar, containing the analytes sorbed (extracted) is placed into an appropriated probe and thermally desorbed, without using the commercial SBSE thermal desorption system. In this work the degradation of esfenvalerate, a pyretrhoid insecticide, was studied by using a chemical oxidation process and the degradation products were monitored by using SBSE probe desorption GC-IT-MS. Degradation was performed by using 50% hydromethanolic solution containing 45 mg L^{-1} esfenvalerate, pH 11.25, 25 mg L^{-1} of hydrogen peroxide, within a 4 hour reaction period. After degradation process, the products were extracted by SBSE by using stir bars (10mm × 0.5mm, 24 µL PDMS coating, Twister, Gerstel) at room temperature (25°C) , for 120 min, with 15% methanol, 12% NaCl and stirring at 1000 rpm. After extraction, the stir bar was placed into the probe of a GC CP 3800 Varian, coupled to an ion trap MS Saturn 2000 (Varian) . Thermal desorption of the analytes were done at 250°C, and the probe heating was held for 18 min. GC analysis were performed on a DB-5 ms fused silica capillary column (30 m x 0.25 mm i.d., 0.5 µm film thickness, Agilent) . The oven temperature was programmed from 70°C (held for 0.5 min) to 300°C/min (held for 6 min) , at 20°C/min. Helium was used as carrier gas at flow rate of 1.2 mL min^{-1}. The MS analyses were done in the scan mode (*m/z* 40 to 450) using electron impact ionization (70 eV) . The temperature of transfer line, ion trap and manifold were set at 300°C, 220°C and 40°C, respectively. By using the SBSE probe desorption-GC-IT-MS method, it was possible to fully identify two products of chemical oxidation of esfenvalerate, 3-phenobenzoic acid and 3-phenoxbenzaldeyde. These compounds were previously described as being metabolites of esfenvalerate, with small estrogenic (endocrine-disrupting) activity and possibly with small environmental impact (McCarthy *et al*., **J. Environ. Monit**. 2006, **8**,197) . The structural elucidation of the other oxidation products of esfenvalerate is still in progress.

Acknowledgements: FAPESP, CNPq

PTh-043
11:10 – 12:20

Adosorption property of PCB 209 congeners by gamma-cyclodextrin polymer

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Keywords:
Atropisomer,Congener specific analysis,GC-MS/MS,PCBs and other toxic substances,Persistent Organic Pollutants (POPs)

Novel aspects:
Nonpolar organic solvents containing PCBs and other toxic substances which passed gamma-CD polymers solid phase were analyzed congener-specifically using the GC-MS/MS.

Abstract:
Polychlorinated biphenyls (PCBs) production in Japan was started by Kaneka Chemical in 1954. The infamous incident of Yusho, rice-oil poisoning, took place in western Japan in 1968. In 1970's, regulations over PCBs production and use became enforced and PCBs disposal measures have been in operation since 1980's. PCBs waste destruction is still going on today. PCBs were widely used as insulating fluids in capacitors and transformers. Although their manufacture and commercial use have been prohibited in many countries since the 1970s because of their strong toxicity, environmental persistence, and bioaccumulation, large amounts of insulating oils contaminated with PCBs are still being used or are kept without being appropriately treated in many countries, including Japan. In the Stockholm Convention on Persistent Organic Pollutants (POPs) , more than 150 countries have agreed to destroy PCBs until 2025. Thus, the efficient and safe treatment of PCB-contaminated insulating oils is a crucial problem from a global viewpoint.
Cyclodextrins (CDs) are a class of cyclic oligosaccharides consisting of several *gamma*- (1,4) -linked D-glucopyranose units. They have a hydrophobic cavity into which a guest molecule of an appropriate size and shape can be incorporated. The ability of CDs to form inclusion complexes with organic molecules has found applications in many areas, including the food and pharmaceutical industries and analytical chemistry. However, in most cases, inclusion complex formation with CDs has been achieved in aqueous media. On the other hand, much less attention has been paid to inclusion complex formation with CDs in nonpolar organic media, because it has been believed that inclusion complex formation in nonpolar organic media would be very difficult due to the unfavorable competition with enormous amount of nonpolar organic solvents against the guest molecules for inclusion into the CD cavity.
We were reported here the removal of PCBs from insulating oil or nonpolar organic solvents by *gamma*-CD polymers as a new adsorbent. Nonpolar organic solvents containing PCBs and other toxic substances were analyzed adsorption charactor which passed gamma-CD polymers solid phase using the GC-MS/MS. PCB 209 congeners were analyzed by product ion obtained by destroying precursor ion using MS/MS method. Other toxic substances were analyzed in the same way. The *gamma*-CD polymers were prepared by the reaction of *gamma*-CD with various kinds of crosslinkers. Among the *gamma*-CD polymers thus obtained, the polymer crosslinked with terephthaloyl units showed the highest adsorption capability towards PCBs. Using this type of polymer (more than 45 wt% of insulating oil or nonpolar organic solvents) as an adsorbent, PCB 209 congeners and other toxic substance, whose initial concentrations were 1 &#65374 ; 100 ppm, were completely removed from isooctane solution.

PTh-044
13:30 – 14:40

Charge exchange ionization in reversed-phase liquid chromatography-atmospheric pressure photoionization mass spectrometry of hormones

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Keywords:
APPI, charge exchange,

Novel aspects:
The use of charge exchange reactions in APPI-MS/MS detection of hormones under reverse-phase HPLC conditions.

Abstract:
Atmospheric pressure photoionization (APPI) has been introduced as an innovative ionization source for LC-API-MS systems to broaden the range of analytes that could be determined using LC-MS even if having weak polarity. From the literature data it is obvious that APPI provides a better ionization for the non-polar analytes than ESI and APCI. Furthermore, photoionization is less susceptible to ion suppression compared to ESI or APCI. Several methods employing APPI-MS detection have been suggested for the analysis of environmentally relevant estrogens, predominantly with toluene as dopant.
Under reversed-phase liquid chromatography conditions, toluene has become a dominant solvent used in the dopant-assisted photoionization (DA-APPI) . Toluene allows charge exchange in solvents with low proton affinities, however, in solvents commonly used in reverse phase chromatography (acetonitrile, methanol) ; ionization via proton transfer prefers to via charge exchange reaction. The use of APPI-MS with a dopant enabling ionization via charge exchange will be evaluated for the determination of seven environmentally relevant estrogens and progestogens (preferably hormones cited in U.S. EPA contaminant candidate list 3) in drinking and river water. Finally, the comparison between APPI-MS using ionization via proton transfer (toluene as dopant) or charge exchange (e.g. anisole, chlorbenzene, brombenzene, 2,4-difluoroanisole, 3- (trifluoromethyl) anisole) will be performed in terms of sensitivity and ion suppression assessment.
The financial support from the Grant Agency of the Czech Republic (Grant Reg. No. GAR P503/10/0975) is gratefully acknowledged.

PTh-045
11:10 – 12:20

Identification of Biodegradation Products of High-Molecular-Weight Perfluorinated Compounds Using Two-Dimensional Liquid Chromatography/High-Resolution Mass Spectrometry

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Keywords:
2 D-LC, Orbitrap, PFCs, biodegradation

Novel aspects:
Two-dimensional liquid chromatography and high-resolution mass spectrometry could demonstrate the occurrence of biodegradation of huge PFCs to small PFCs in aerobic biodegradation.

Abstract:
Perfluorinated compounds (PFCs) have been considered an environmental problem. Early studies on PFCs focused on the presence of perfluoroalkyl carboxylic acids (PFCAs) and sulfonic acids (PFSAs) in environmental matrices and their possible biological effects. However, more complicated PFCs were usually used rather than simple molecules such as PFCAs and PFSAs. There is limited information about such complicated PFCs. Although major fluorochemical companies now have phased out of production and use of PFOA, PFOS and PFOS-related products, PFOA and PFOS still have been found in various contexts. Both fluorochemical products and environmental samples always contain complicated matrices. It is difficult to determine what kinds of PFCs really exist.

In the past few years, two or multidimensional liquid chromatography has been attractive for analyses of complex mixtures. Multidimensional chromatography coupled with mass spectrometry notably offers comprehensive analysis and has been applied to the characterization of natural products and industrial materials. In this study, we used an off-line 2 D liquid chromatograph coupled with a high-resolution mass spectrometer (2 D-LC/HR-MS) to identify commercial fluoroproducts and their biodegradation products.

Fluoroproducts examined consisted of four products. Three of them were products sold before the regulation. All of them were purchased in Japan. Liquid samples were diluted in water by 3000-fold. Activated sludge was added to the diluted samples at a concentration of 30ppm. The samples were transferred in closed containers, which were made to be able to supply oxygen for aerobic biodegradation. Ten-mililiter portions of samples were taken from each stirred sample once a week for a month. The portions passed through solid phase extraction cartridges, OASIS WAX (Waters) , to extract PFCs. PFCs were eluted from the cartridges with 0.1 % NH_4OH methanol. The eluates were analyzed by liquid chromatograph/mass spectrometer, Ultimate 3000/ Exactive (ThermoFisher) .Ultimate 3000 and Exactive were used for acquisition of mass spectra with a high resolving power of 100,000. In addition, Acquity and Xevo TQ (Waters) were used for quantitation of PFCAs and PFSAs and acquisition of product ion spectra. Two-dimensional LC was carried out with two different kinds of columns. A TSK-Gel ODS-100S from TOSOH and an Epic-FO column from ES-industries were used for chromatography.

Acquired mass spectra with single LC included numerous ions. The interpretation of mass spectra was, therefore, very difficult. Two-dimensional LC could effectively separate these compounds depending on the interaction strength with two different types of columns. The mass spectra that generated from separated compounds were easier to interpret than the spectra from unseparated compounds. Fragment ion spectra were also acquired.

The chemical structures were determined from the interpretation of the data of samples before biodegradation. A dominant ion with an *m/z* value of 1574.2809 was found in one product examined. Because three fragment ions that had equal spaces of 357.007 were found in the observation with collision gas, the compound was the molecule that had three $C_4F_9SO_2NCH_3C_2H_4O$ groups. If triisocyanate such as Tolonate reacts with an alcohol, $C_4F_9SO_2NCH_3C_2H_4OH$, the chemical formula of the molecule yielded will be $C_{45}H_{60}N_9O_{15}S_3F_{27}$. The exact mass of the ion is 1574.2867. The two values were well-matched.

Biodegradation products were also examined by analyses of the samples after biodegradation. Two out of four products examined showed unequivocal increase of concentration of PFCAs. Although the above-referenced sample did not show any increase of PFCAs and PFSAs, a degradation product that had a carboxyl group at the terminal was identified. The result complemented the presumption of original structure. However, further degradation structures such as sulfonamide were not found.

Two-dimensional LC/HR-MS demonstrated the occurrence of biodegradation of huge PFCs to small PFCs in aerobic biodegradation. We need further investigation of fluoroproducts that have been used so far.

PTh-046
13:30 – 14:40

Current status of organophosphorus compounds contaminants in Maizuru Bay, Japan

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Keywords:
organophosphorus compounds, water, sediment, mussel

Novel aspects:
Organophosphate esters and pesticides were detected in water, sediment and mussel samples from Maizuru Bay, Japan.

Abstract:
Organophosphorus (OP) compounds which have been utilized as flame retardants, plasticizers, stabilizers, antifoaming and wetting agents, and additives in lubricants and hydraulic fluids etc. are the useful chemical compounds and are used in the various areas. It is well known that aquatic environment has contaminated by volatilization, leaching or abrasion from the broad application range of these compounds. In this study, the concentrations of eight species of organophosphate esters (OPE) and OP pesticides (diazinon, fenitrothion, chlorpyriphos and iprobenphos) are measured in water, sediment and green mussels from Maizuru Bay and the distribution of these compounds in water environment is discussed.
Water sediment and mussel samples in Maizuru Bay were taken from July 7th to 15th, 2009. Subsurface water samples were taken in 7 sites and the surface sediment samples (0 5 cm) were taken using a Ekman-Birge type bottom sampler in 13 sites. The mussel (*Mytius galloprovincialis*) samples were taken at 9 sites. The shell lengths of the green mussels were in the range of 380 - 750 mm. Three mussel samples in each site were homogenated before analysis. Water samples were stored in a fridge at 3 °C and their samples were analyzed within a few day. Sediment and mussel samples were stored in a freezer at -20 °C for until chemical analysis.
Water samples were extracted with dichloromethane by shaking for10 min. The aqueous layer was dried with anhydrous $Na_2SO_4$and was concentrated up to 0.5 ml after the addition of Atradine-d_{25} as an internal standard. The analytes were measured by GC-MS. OPs in sediment and mussel samples were extracted with acetone. After centrifugation, the supernatants were cleaned by addition of distilled water, celite and zinc acetate. After removal of suspended matters by filtration, the analytes in liquid layer were extracted dichloromethane. After the addition of Atradine-d_{25}, the organic layer was concentrated up to 0.5mL. The analytes were measured by GC/MS.
The distribution of OP compounds in Maizuru Bay was surveyed. The concentrations of OP in water samples from Maizuru Bay were in the range of 2.7 62 ng/L, which these level was under a thousandth of acute toxic level for aquatic organisms. The concentrations of OPEs were high the order of TBXP > TDCPP >TCEP > TBP > TCP > TPP > TEP. This trend is similar to those in 1976-1996. Diazinon, fenitrothion and chlorpyriphos of OP pesticides were detected in water samples.
The concentrations of OP compounds in sediment from Maizuru Bay were in the range of <0.5 - 56 ug kg^{-1} dry weight (dw) . The patterns of OPEs in sediment are classified two groups. The patterns of OPEs concentrations in a group are characterized that OPEs concentrations are high the order of TBXP > TDCPP or TBP, and the other OPEs are similar concentrations. The patterns of OPEs concentrations in the other group characterized that TDCPP concentrations is the highest among OPEs. Diazinon was detected in all sediment samples from Maizuru Bay and the concentrations of diazinon were in the range of 1.8 and 71 ug kg^{-1} dw. The detection frequencies of fenitrothion and chlorpyriphos were low in sediment.
The concentrations of OP in mussel from Maizuru Bay were in the range of <0.5 and 34 ug kg^{-1} wet weight. The concentrations of OPEs were high the order of TBP > TDCPP >TCP >TBXP = TPP = TCEP.
The partition rate between water and sediment (Kws) of diazinon and fenitrothion were in the range of 200 and 1300, and in the range of 200 and 300, respectively and the partition rate between water and biological samples (Kwb) of diazinon and fenitrothion were in the range of 700 and 3300, and in the range of 450 and 700, respectively, suggesting that these pesticides prefer biota to sediment.

PTh-047
11:10 – 12:20

Tandem SPE clean up/extraction: strategy to minimize matrix effects on LC-MS determination of endocrine disrupters and pharmaceuticals in sewage samples

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Keywords:
Emerging Contaminants；Matrix Effects；Sewage

Novel aspects:
The work uses tandem SPE (SAX+HLB). This approach was efficient at removal of LAS and to minimize their matrix effects on ESI responses of analytes in sewage samples extracts.

Abstract:
In recent years, a variety of organic compounds, also called emerging contaminants, have being detected by LC-MS, at low concentrations (ng L-1), in samples of surface water, wastewater, groundwater, and even drinking water [1-3]. According to Taylor [4], the matrix effect is the "Achilles' heel" of the HPLC-MS techniques. Therefore, it is extremely critical to evaluate and/or minimize the influence of complex matrix composition on the HPLC-MS responses of analytes. Few articles have evaluated thoroughly the matrix effect in analysis of emerging contaminants in sewage samples. In this work a new method for the determination of three endocrine disrupters (estradiol, ethinyl estradiol, and bisphenol A) and five pharmaceuticals (sulfamethoxazole, trimethoprim, diclofenac, bezafibrate and miconazole) in raw and treated sewage samples was developed and validated. The method consisted of the application of tandem SPE procedure that uses both a strong ion exchange sorbent (SAX) and a modified divinylbenzene-pyrrolidone sorbent to reduce the levels of linear alkylbenzenesulphonate (LAS) and to concentrate the analytes of interest from sewage samples, prior to analysis by liquid chromatography combined with high-resolution mass spectrometry (LC-HRMS). The influence of matrix composition on the ionization efficiency, the SPE recoveries, and the sensitivity of the method was determined. The SAX sorbent was capable of significantly removing LAS content in sewage samples extracts. It was also capable of retaining the analytes that were eluted with ethyl acetate with recoveries that varied from 17 to 35 % (CV < 9 %). This approach was very efficient at minimizing the matrix effects. The sum of the recoveries from both sorbents varied from 30 to 70% (CV < 9 %). The mass spectrometry detection was performed using a LC-ESI-IT-TOF/MS instrument working at high-resolution (10.000 FWHM) and high mass accuracy (< 5 ppm). The instrumental limits of quantitation varied from 0.4 µg L-1 to 3.3 µg L-1. The method limits of quantitation ranged from 3.3 ng L-1 to 41 ng L-1. The method was successfully applied to the determination of analytes in raw sewage samples at the Arrudas Sewage Treatment Plant, Belo Horizonte, Brazil and also to evaluate the efficiency of different experimental sewage treatment systems.

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PTh-048
13:30 – 14:40

Simultaneous analysis of anionic, amphoteric and non-ionic surfactants using ultra-high speed LC-MS/MS

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Shimadzu Corporation., Kyoto, Japan

Keywords:
surfactant, LAS, Betain,Heptaethyleneglycoldodecylether,TQ

Novel aspects:
Simultaneous analysis method of anionic, amphoteric and non-ionic surfactant using ultra-high speed polarity switching technique of LC-MS/MS was developed.

Abstract:

Introduction

Surfactant chemistry has made a considerable impact on a number of household products including detergents, shampoos and toothpaste. Products are generally classified by the type of each hydrophilic substructure into anionic, cationic, amphoteric and non-ionic surfactants. Either anionic or non-ionic surfactants are typically used as synthetic detergents, however, to better elucidate the potential risk in environmental samples, mainly in agricultural soils and sediments, methods need to take into account a range of surfactant chemistries. Current surfactant monitoring methodologies tend to focus on a specific surfactant. Here, we have developed the simultaneous analysis method for typical anionic, amphoteric and non-ionic surfactant using LC-MS/MS.

Method

Commercially available surfactants were used for this experiment. Standards of surfactants were diluted with water to an appropriate concentration and then determined by LC-MS/MS. As an LC-MS/MS system, UHPLC was coupled to triple quadrupole mass spectrometer (Nexera MP with LCMS-8030, Shimadzu Corporation, Kyoto, Japan) . Separation was achieved using a YMC-Triart C 8 column (100mmL., 2.0mmI.D., 1.9um particles) and column oven temperature was maintained at 40 C. Samples were eluted at flow rate 300uL/min with a binary gradient system ; the mobile phase consisted of (A) 10mM ammonium acetate buffer and (B) mixture of 10mM ammonium acetate / acetonitrile / isopropanol (1 / 4 / 5) . LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode with ultra-fast polarity switching.

Preliminary data

The following standard surfactants were selected and analyzed ; anionic surfactant : linear alkylbenzene sulfonate (LAS) C10-C14 mixture, amphoteric surfactant : EMPIGEN BB Detergent (Betaine) C10, C12, C14 mixture and non-ionic surfactant : heptaethyleneglycoldodecylether (HEDE) . Full scan measurement by flow injection analysis (FIA) was conducted to determine the optimum ionization polarity of target compounds followed by MRM transition optimization by FIA. As a consequence, all LASs were detected as the de-protonated ions and m/z 183 was selected as the product ion of MRM transitions for all LASs (C10-C14) . All Betaine were detected as protonated ions and m/z 104 was selected as the product ions of MRM transitions for all Betaine (C10, C12 and C14) . HEDE yielded the protonated ion as the precursor and m/z 133 was selected as product ion for MRM transition. As compounds selected in this experiment formed either positive or negative ion, high-speed polarity switching is an important parameter to consider in developing an optimized method. The dilution series of these compounds were analyzed and all compounds were detected at sub ppb level with excellent linearity. In addition, the quantitative analysis of real world sample using the kitchen detergents and liquid soap was achieved using this method

PTh-049 Limitations of a commercially available plasma air purifier

11:10 – 12:20

Stefan Schmid[1], Andreas Gerecke[2], Herbert Haechler[3], Hubert Hilbi[4], Simon Weidmann[1], Renato Zenobi[1]

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Keywords:
Non-thermal plasma, Cleaning efficiency, Air-cleaning

Novel aspects:
Efficiency study of a commercially available plasma air purifier over a wide range of different substances

Abstract:
The molecular processes in a commercially available AC, driven plasma air purifier (PAP) were studied in detail. Such air purifiers are supposed to break down all air contaminants to small, nontoxic molecules (e.g. H_2O and CO_2) . However, the degradation mechanisms in such PAPs are not known yet. In this study, we examined the exhaust of a commercially available PAP to determine its efficiency and the molecular processes taking place. Three different classes of substance were studied : environmental toxins representative for of low MW molecules, a high-mass protein, and various bacteria to represent very high masses. One goal of this project was to examine the limitations of such commercially available air purifying systems.
A setup was designed in such a way that the PAP could be studied under realistic conditions, simulating common heating and ventilation systems. Phenanthrene and methyltriclosane were chosen as small molecules. Bovine serum albumin (BSA) was chosen as a model high mass protein. *Legionella Pneumophila* and *Bacillus anthracis* were used to cover the class of airborne infectious agents. The sampling method was adapted to the respective compounds. Adsorption tubes were used for the low MW molecules ; bubbling 10% of the PAP exhaust through water was used to quantify the amount of degraded BSA ; *L. pneumophila* and *B. anthracis* were sampled using agar strips, which were afterwards wrapped in sterile containers and incubated.
The study of environmental toxins using GC-MS showed a degradation of 31.0% and 16.9% for phenanthrene and methyltriclosane, respectively (relative error, 13 %) . However, no characteristic degradation products could be found. Therefore, a mass balance with methyltriclosane was conducted which yielded surprising results. On the 4 copper electrode surfaces of the PAP exactly 17% were methyltriclosane found. Since the degradation was determined by performing an experiment with and without activated PAP, the decreased amount of methyltriclosane was considered as degraded. However, our experiments do not support the hypothesis of degradation for small molecules anymore.
Measuring the PAP degradation performance of BSA showed a reduction of 81.1±30 %, when comparing experiments with and without the plasma activated. The copper electrodes showed a white film after the experiment with the plasma activated. After dissolving the white precipitate in water, LC-MS and MALDI-MS experiments identified it as BSA. 1 % of white and water insoluble crystals which were identified as polymerized BSA using MALDI-MS equipped with a high mass detector. The reduction in amount of various bacteria showed, that the PAP is capable of reducing aerosolized bacteria in air. However, it seems that the reduction is mainly occurring due to adherence to the copper electrodes.

PTh-050
13:30 – 14:40

Multi-component quantitative analysis of pharmaceuticals and personal care products in the environment by LC-MS/MS with fast polarity switching

Natsuyo Asano, Kiyomi Arakawa, Shinjiro Fujita, Manabu Ueda, Kazuo Mukaibatake, Ichiro Hirano

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Keywords:

LCMS-8080, higher sensitivity, multi-component quantitative analysis , fast polarity switching, and pharmaceuticals and personal care products (PPCPs)

Novel aspects:

Fast polarity switching results have been equivalent to dedicated single polarity experiments for the analysis of PPCPs in environmental samples.

Abstract:

Pharmaceuticals and personal care products (PPCPs) constitute a group of emerging contaminants which have received considerable attention in recent years. Monitoring of PPCPs in the environment is vital as many of these compounds are ubiquitous, persistent and biologically active with recognised endocrine-disruption functions. Given the hazardous nature of these compounds, there is a need to provide fast and sensitive multi-residue methods that are able to analysis multiple classes of compound within one analytical procedure. Here we report a new multi-residue UHPLC-ESI-QqQ method that utilises fast polarity switching with an optimised chromatographic gradient that removes matrix effects and results in excellent ng/L detection levels. Furthermore, we evaluate the performance of polarity switching in comparison to dedicated single polarity experiments.

Natural river and lake water was collected from the Shiga region (Japan) and spiked, without any sample pretreatment, at a range of concentration levels (1 - 10000 ng/L) with 15 PPCPs (e.g. Carbamazepine, Dehydronifedipine, Gemfibrozil, Triclocarban) . Separation was achieved using a Shim-pack XR-ODS III column (50 x 2.0 mm, 1.6 µm) maintained at 40° C on a UHPLC system, Nexera (Shimadzu, Japan) . The method was maintained at a flow rate of 0.4 mL/min with mobile phase A : water/0.1 % formic acid and B : acetonitrile. The gradient (detailed in preliminary data) was optimized to minimise matrix effects. A higher sensitivity triple quadruple mass spectrometer, LCMS-8080 (Shimadzu, Japan) operating in SRM mode with fast polarity switching (20 msec) was used for the detection of positively and negatively charged analytes.

As a result of the complex matrix PPCPs are present ; the occurrence of ion suppression/enhancement is commonly described in literature and results in reduced MS/MS detection limits and inferior precision. For this reason, a gradient was optimised that focused target analytes at the head of the chromatographic column while allowing the interfering environmental matrix to be eluted ; this resulted in excellent recoveries of around 100 % for almost all studied compounds. This was achieved using a gradient which held the aqueous mobile phase at 100 % for 6 min, and subsequently increased the organic mobile phase to 80 % over 10 minutes and then to 100 % organic mobile phase.

PPCPs encompass a wide range compound classes and chemical properties and consequently it is necessary to employ both positive and negative electrospray ionization for complete analysis in a single run. All compounds were measured by SRM with fast polarity switching (20 msec) for multi-component analysis. Excellent limits of quantification were achieved in the range 1 - 50 ng/L for nearly all studied compounds, with outstanding linearity (R^2> 0.999) .

To evaluate the capability of polarity switching the data quality obtained was compared to dedicated negative or positive analysis. Comparisons were made by selecting compounds that prefer positive (Carbamazepine and Dehydronifedipine) and negative ionisation (Gemfibrozil and Triclocarban) . Findings showed that the data quality obtained during polarity switching experiments was directly comparable to that achieved during dedicated positive or negative analysis. In addition, long term stability was investigated by making 100 injections over >10 hours, with polarity switching presenting excellent stability.

PTh-051
11:10 – 12:20

Screening and library search of Environmental pollutants in Japanese environmental water using LC-MS/MS

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Keywords:
PPCPS Environment LC/MS/MS Water

Novel aspects:
Fast Aqcuisition time, High Sensitivity, Direct measurement, Over 100 compounds

Abstract:
Introductions
In recent years, many kinds of chemical substances are polluting environmental water. When people take a medicine, use insect repellent, or apply cosmetics, these chemicals flows into the water in the environment, finally. These compounds are called PPCP (Pharmaceuticals and Personal Care Products) as a whole. Furthermore, it is known that the pesticides originally used for agricultural products are polluting environmental water. These compounds are also contained in PPCP in a broad sense.
The diversity and the numerousness of these compounds make method development challenging. But In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is able to analyze polar, semi-volatile, and thermallylabile compounds covering a wide molecular weight range, such as pesticides, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products etc. In addition, state-of-the-art LC-MS/MS instruments operated in selective Multiple Reaction Monitoring (MRM) mode, offer unmatched selectivity and sensitivity to quantify PPCP reproducibly at trace levels without time consuming and extensive sample preparation.
AB SCIEX QTRAP®4500 LC/MS/MS System can measure many compounds simultaneously because of its fast MRM scanning, and its can measure compounds high-sensitive because its hasQ-jet® 2 ion guide.
More recently, it is discussed that detection only by MRM cause false positive and false negative. To avoid false detection, one useful process is library search for a fragment ion spectrum. This process judges positive or negative by comparing the spectrum of standard with the spectrum of the compound detected in the sample. At 1 ch MRM, while judging from one fragment ion, by the library search using a spectrum, a judgment is performed from all fragment ions which the compound has. Therefore, library search can do more precise judging.
ABSCIEX QTRAP® 4500 LC/MS/MS System can acquire fragment ion spectrum fast and sensitive because its has Linear Ion Traptechnology with Linear Accelerator™ Trap. And AB SCIEX has fragment ion spectrum library that contains large number of compounds.

LC-MS/MS is suitable for measurement of PPCP, and judgment by library search is desirable. Therefore, AB SCIEX QTRAP® 4500 LC/MS/MS System suitable for PPCP screening.

Methods and results
20 Japanese environmental water samples were injected directly into AB SCIEX QTRAP® 4500LC/MS/MS System to detection PPCP at low parts-per-trillion (ppt) levels (ng/L) . 4500 quick scanning speeds supported simultaneous analysis of 304 compounds (273 for positive, 31 for negative) were acquired by using MRM. Enhanced Product Ion Scan (EPI) was used for acquiring the fragment ion spectrum at ppt levels, and "Mega-library " spectrum library was used to judge compounds detected by MRM were true or false. EPI is high-sensitive and high-speed fragment ion scan mode, supported by Linear Ion Traptechnology with Linear Accelerator™ Trap. "Mega-library " is fragment ion spectrum library that contains over 1240compound's spectrums.
From Japanese environmental water, some compounds were detected by MRM. The detected compounds did not related sampling point. According to results of the library search, some compounds were truly detected, but some compounds were false positive.
As a conclusion, the PPCP exist Japanese wide area environmental water. So it is necessary to measure much more samples to know a trend, such as regionality.

PTh-052
13:30 – 14:40

OPTIMIZATION OF SOLID PHASE EXTRACTION FOR PERFLUORINATED COMPOUNDS ANALYSIS IN WATER SAMPLE

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Keywords:
Micropollutant, Perfluorinated compounds (PFCs) , PFOA, PFOS, Solid Phase Extraction

Novel aspects:
The solid phase extractionwas optimized for analysis of twelve PFCs in water sample, especially tap water and river water (with low matrix) .

Abstract:
In recent years, perfluorinated compounds (PFCs) have appeared as a new class of global contaminants. Researchers have reported PFCs contamination in surface water, tap water and bottled water around the world. They are particularly difficult to deal with once released into the environment, because they do not break down easily, they can travel long distances carried by air or in water and they can accumulate in human and animal tissue. To understand the sources and fate of these compounds, it is essential to optimize the analytical methods for a wide range of perfluorinated compounds in water sample. Solid phase extraction (SPE) coupled with high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) is generally used to analyze PFCs in environmental matrices. Researchers have reported the difficulties in analysis of short chain (C 4 C 6) and long chain (C10 C12) PFCs. The ineffective SPE procedure and matrix interferences were the two major difficulties for analyzing environmental water sample. To overcome these problems, the optimization of SPE process is needed. The objective of this study was to evaluate the optimum SPE condition for two ion exchange (OasisWAX and PresepPFC-II) cartridges for analyzing 12 PFCs in water sample. These cartridges were also compared with PresepC-Agri (C_{18}) and OasisHLB, which are generally used for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) analysis. Several options for optimizing SPE were evaluated such as adjusting sample loading flow rate, washing sample's bottle by methanol, optimizing cartridges drying step, adding matrix washing step, conducting sequential elution, adding volume of solvent and optimizing nitrogen purging. Ion-exchange cartridges (OasisWAX and PresepPFC-II) were showed better result comparing to both PresepC-Agri (C_{18}) and OasisHLB. Flow rate 10 mL/min showed better recoveries of most chemicals comparing to flow rate 5 and 20 mL/min. Comparison of drying cartridges and without drying cartridges was conducted for both PresepPFC-II and OasisWAX. The average increase of all twelve chemicals was 9 %. An experiment was conducted for washing the sample bottles two times after loading to the cartridges. Five milliliter of methanol (2 times) was applied for each washing. Recoveries of C10 C12 increased by 2 %, 12% and 33%, respectively for both cartridges. The elution was done three times by using 2 mL 0.1%NH 4 OH in methanol each time. More than 97% of all compounds were eluted in the first elution. An experiment on the effect of PFCs lost during nitrogen purging step was also examined. There was no significant in the loss of PFCs during nitrogen purging for 1 hr, 2 hrs and 3 hrs. The solid phase extraction was optimized for analysis of twelve PFCs in water sample, especially tap water and river water (with low matrix) . Coupled with the use of HPLC-MS/MS, a method detection limit in the range of several tens of parts-per-quadrillion (pg/L) in water can be achieved.

PTh-053 Analytical method and homolog distribution of OH-PCBs in ambient Air

11:10 – 12:20

Yuki Haga[1], Motoharu Suzuki[2], Masahiro Tsurukawa[1], Yoshiya Yamoto[3], Chisato Matsumura[1], Takeshi Nakano[4]

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Keywords:
OH-PCBs, air, GC/MS

Novel aspects:
OH-PCBs in ambient air was detected using QFF and PUF which were set to Hi-Volume air sampler.

Abstract:
This analytical method is suitable for the determination ofHydroxylated Polychlorinated Biphenyls (OH-PCBs) in ambient air by gas chromatography- high-resolution mass spectrometer with selected-ion monitoring (GC/HRMS-SIM) . OH-PCBs are considered as one of the endocrine disturbing chemicals because OH-PCBs have negative impacts on the some thyroid and female hormones. OH-PCBs are formed by metabolism of PCBs by the cytochrome P450 enzyme-mediated oxidation and OH radical reaction of PCBs. The concentrations of OH-PCBs in the environmental waters, air, sediments and living things have been investigated and OH-PCBs have been detected from every medium. In Canada, OH-PCBs were also detected from the rain and snow. In this study, we modified the analytical method and investigated the concentration of OH-PCBs in ambient air. The air was introduced into quartz fiber filter (QFF) and poly urethane foam (PUF) which were set to Hi-Volume air sampler at a flow rate of 700 L/min for 24 hr (total volume was 1000 m^3) . After collection was completed, clean-up spike (OH-Di-Hp-CB-$^{13}C_{12}$ 3.0ng) was added to the PUF. Both QFF and PUF were simultaneously extracted with acetone by ASE. The acetone extract was added 6 mL of 5 % sodium chloride solution, and extracted with 2 mL of hexane, twice. The hexane phase was clean upped with pre-washed Sep-Pak Plus Florisil. Sep-Pak Plus Florisil was eluted with 0.5% diethyl ether/hexane 8 mL (for PCB) , and then with 50% acetone/methanol 10 mL (for OH-PCBs) . The eluate was concentrated until just before dryness. After derivatization and alkaline digestion, added 6 mL of 5 % sodium chloride solution, and extracted with 2 mL of hexane, twice. Concentrated to about 1 mL and dehydrated with anhydrous sodium sulfate. The concentrate was applied to a pre-washed Sep-Pak Plus Florisil, and eluted with 8 mL of 5 % diethyl ether/hexane. The eluate was concentrated to100 μL and added syringe spike (MBP-70-$^{13}C_{12}$ 0.50 ng) . The method detection limits (MDL) and the method quantification limit (MQL) were 0.067 - 0.13 and 0.17 - 0.33 pg/m^3, respectively. The average of recoveries (n = 3) from 1000 m^3 of air sample added with 3.0ng OH-PCBs were almost 60-110 % , and the relative standard division was 1.2 - 24% . Lowly chlorinated OH-PCBs tend to be collected in PUF and highly ones tend to be collected in QFF. In the air samples, OH-PCBs were detected 1 pg/m^3 order and the lowly chlorinated OH-PCBs were dominant.

PTh-054
13:30 – 14:40

Application of combined UPLC-TOF-MS and combustion ion chromatography for electrolytic degradation mechanism of PFOS in water

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Keywords:
Activated-carbon, bond cleavage, byproducts, TOF-mass

Novel aspects:
Combined UPLC-TOF-MS and combustion ion chromatography technique used for first time in elucidating electrolytic degradation mechanism of PFOS.Carbon-carbon bond cleavage adjacent to sulfonate functional group is the first reaction step.

Abstract:
Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the most extensively investigated representative compounds of perfluoroalkyl carboxylate (PFAC) and perfluoroalkyl sulfonate (PFAS) groups respectively. PFOA can be degraded by UV photodegradation in water while PFOS is extremely resistant to the degradation. But the latter in water can be degraded very easily by electrolytic method than by UV photodegradation. However, electrolytic degradation mechanism and pathways for PFOS are not still known.

Mechanism of electrolytic degradation of PFOS in water is investigated and discussed for the first time using ultra-pressure liquid chromatography time of flight mass spectrometry (UPLC-TOF-MS) combined with combustion ion chromatography. Batch tests for electrolytic degradation (current = 1.0 A, DC voltage : 33.0 V, surface area of circular platinum electrodes = 236 mm2, current density = 4.23 A/m 2) of PFOS (concentration = 1.0 mg/L, volume = 250 ml) were conducted using a cylindrical air-tight and heat-resistant glass vessel for 60 min durations. Sodium bicarbonate (50.0 mmol/L) was added to the reaction solution for enhancing electrical conductivity. Liquid samples were collected and analyzed (UPLC-TOF-MS) for PFOS and its degradation intermediates. Head space gas samples were adsorbed to activated carbon columns by continuous nitrogen gas flow through the space. Total fluoride ion concentration and short carbon-chain intermediates in the sample (i.e. activated carbon after head space gas adsorption) were analyzed using combustion ion chromatography. PFOS degradation mechanism in water is discussed based on the identified intermediates.

Though it has already been demonstrated that fluorinated short carbon-chain byproducts of PFOA degradation remain mostly in gas phase (i.e. head-space gas) , no such products of PFOS electrolytic degradation in activated carbon columns were observed in this investigation. Absence of fluorinated short carbon-chain compounds and additional fluoride ion contents due to oxidation of the compounds during combustion ion chromatographic analysis strongly suggested that either the compounds were actually absent during PFOS degradation or the compounds in gas phase were not adsorbed to activated carbon. This point still remains to be elucidated. Analysis of water samples by UPLC-TOF-MS showed fragments (with seven carbon atoms) of PFOS having hydroxyl functional group suggesting carbon-carbon bond cleavage adjacent to sulfonate functional group followed by its hydrolysis during electrolytic degradation of PFOS. The present investigation needs to be continued further to understand whether fluorinated short-carbon chain byproducts of PFOS electrolytic degradation do not really exist in head-space gas and elucidate on complete degradation mechanism.

PTh-055
11:10 – 12:20

2,3,7,8-tetrachlorodibenzo-p-dioxin congener in breast milk among 3 hot spots in Vietnam

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Keywords:
TCDD, dioxin congener, hot spot, Vietnam

Novel aspects:
Investigating dioxin and furan congener profile in 3 dioxin - contaminated areas in VIetnam, which are known as hot spots, we found TCDD contribution were different in these hot spots.

Abstract:
Using GC-MS to quantify 7 congener of polychlorinateddibenzo-p-dioxins (PCDDs) and 10 congener of polychlorinated dibenzo-p-furans (PCDFs) in breast milk of Vietnamese primiparous mothers who are living in dioxin-hot spots in Vietnam. These areas are former United State Airbases in Southern Vietnam, where herbicide was stored during the Vietnam War. These airbases are knowns as hot spots because of extremely higher level of dioxin in comparison with sprayed and non-sprayed areas. There major hot spots are Bien Hoa, Da Nang and Phu Cat. 52 breast milk samples in Bien Hoa (BH) , 43 in Da Nang (DN) , and 23 in Phu Cat (PC) , and 19 in Kim Bang (KB) - a non-exposed area were collected between 2008 and 2010. Mean total toxicity equivalence (TEQ-WHO 2005) of PCDDs/PCDFs in BH, DN, PC were 10.9, 16.0, 14.8 pg-TEQ/g lipid, respectively while it was 4.51 pg-TEQ/g lipid in KB. Mean of TCDD concentration in BH, DN, PC and KB were 3.60, 2.47,1.79 and 0.64 pg/g lipid, respectively. DN and PC have same congener dioxin and furan profile but different from BH. In DN and PC the relative abundance of 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD) is about 2 - 3 times higher than 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) ; however relative abundance of TCDD is higher than that of PeCDD in BH. When TCDD concentration were categorized, percentage of samples have TCDD in the highest category (>10 pg/g lipid) or lowest category (< 1 pg/g lipid) in BH are higher than PC and DN, which mean that there is a large variation in TCDD level from BH, especially a small number of mothers in BH are highly exposed. Mean percentage of TCDD contribution to TEQ in BH, DN, PC and KB were 24.6 % , 15.1 % ,12.2 % and 13.3 % , respectively. Various herbicides were used during the Vietnam War including Agent Orange, Agent Purple, Agent White, Agent Blue, Agent Pink.These herbicides were contaminated with different concentration of TCDD, which may explain the different in TCDD levels from those hot spots.

PTh-056
13:30 – 14:40

Profiling waters from natural sources and areas of oil sands activity using Fourier transform ion cyclotron resonance mass spectrometry

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Keywords:
Fourier transform ion cyclotron resonance mass spectrometry, atmospheric pressure photoionization, environment, oil sands

Novel aspects:
Usage of APPI and FTICR mass spectrometry for the characterization and comparison of organic components, including compounds of low polarity, found in waters from different sources in the Athabasca region

Abstract:
Due to pressures on a finite supply of petroleum as consumption continues to grow, it is necessary to turn to less conventional sources, such as oil sands. The Athabasca oil sands are located in Alberta, Canada, and consist of clay, sand, water, and bitumen. An alkaline hot water extraction process can be used to separate the bitumen, which can then be upgraded to synthetic oil. Approximately three barrels of water are consumed during the production of one barrel of oil, resulting in intensive water usage by the oil sands industry. This oil sands process water (OSPW) must be stored in vast tailings ponds, as there is a zero discharge policy, and there is a need to monitor potential effects upon local water quality. It is important to be able to differentiate between those organic components found in the aquatic environment due to natural processes, such as expected seepage of oil sands material, and those which arise due to human activity.

Samples were acquired from a range of natural water and oil sands process water sites in the Athabasca Basin and analysis was performed using a 12 T solariX Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) . Previous research into the characterization of OSPW has typically relied upon the usage of electrospray ionization (ESI) , usually due to the targetting of naphthenic acids within the complex mixtures. Whilst ESI is best suited to the study of polar and ionic species, atmospheric pressure photoionization (APPI) can be applied to the study of less polar species and can produce radical ions in addition to protonated/deprotonated ions. ESI and APPI represent complementary methods, where APPI is an amenable ionization method for the study of a broad range of compounds, such as less polar, sulfur-containing compounds and hydrocarbons which do not incorporate heteroatoms. Mass spectra of complex mixtures that have been acquired using APPI are typically more complex, due to the greater number of components observed and the fact that radical ions are observed in addition. High field FTICR mass spectrometry offers ultra-high resolving power and mass accuracy, which afford high confidence in assignments of species within complex mixtures, which is particularly important for mass spectra generated using APPI.

Following assignments of elemental compositions, it is possible to visualize the data using different methods of categorization, such as heteroatom content, carbon number, “hydrogen deficiency “ (Z) , or double bond equivalents (DBE) . Principal component analysis (PCA) has been used to highlight similarities and differences between water sources. Differences were found between river waters, ground waters, and OSPW, indicating potential for determining anthropogenic influences on the aquatic environment.

PTh-057 Analysis of biomarkers of the pesticide imazalil using LC/MS/MS.

11:10 – 12:20

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Keywords:
Biomarkers, Pesticides, LC/MS/MS, Quatification, Urine

Novel aspects:
This study presents a new quantitative method for quantification of the pesticide imazalil and its major metabolite DCPI in human urine using LC/MS/MS.

Abstract:
Introduction - Imazalil is a widely used post-harvest fungicide applied on a variety of crops, especially on fruits. Imazalil is also used in veterinary medicine as an antimycotic drug.
Imazalil is classified as "Likely to be carcinogenic inhumans, " according to EPA. Exposure to imidazole derivatives is also suspected to produce craniofacial malformations in vertebrate development. Studies on rats have shown that imazalil is metabolized into 25 different metabolites with 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)-1-ethanol (DCPI) as the major metabolite. There are very few studies in humans, however a case study of a patient treated with imazalil indicated a half-time of 2 h in serum. Various methods have been published for determination of imazalil residues in agricultural and food products. However, no methods have been described for determination of imazalil or its metabolites in human biological samples. Human occupational exposure can be substantial especially in developing countries were the use is high. It is especially common that women in fertile age are exposed during post-harvest treatment. Thus, methods for measurement of biomarkers of exposure is valuable. The aim of this study was to develop a simple liquid chromatography-tandem mass spectrometry (LC/MS/MS) method for the analysis of imazalil and DCPI in human urine.
Method Several SPE columns were evaluated and optimized for sample pretreatment. The eluates were analyzed using electrospray ionization and selected reaction monitoring (SRM) in positive ion mode after separation on a C18 LC column. The mobile phase used was water and methanol with0.1% formic acid. D_5-labeled imazalil was used as an internal standard.
Results Two SPE columns could be used for samples pretreatment, C2 and Oasis HLB. The sample was retained at neutral pH and eluted using 1% formic acid in methanol. For quantification of imazalil the transition atm/z 297.1-159.0 and for DCPI the transition m/z 257.0-69.2 was used. The method have an LOD of 1 ng/ml for both imazalil and DCPI. Data on levels found in a population will be presented.

PTh-058
13:30 – 14:40

Mass spectrometric investigation of mechanisms for methane formation from epicuticular wax under aerobiosis and UV

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Keywords:
GCMS, photolysis, methane , greenhouse gases

Novel aspects:
The combination of GCMS analyses snd photolytical studies has resulted in detailed understannding of the underlying mechanisms for aerobic formation of methane from plants.

Abstract:

Introduction

Methane (CH_4) is the second most important long lived greenhouse gas. The total global CH_4 source strength is well described, whereas substantial uncertainties exist about the strength of individual source components. When it was discovered that terrestrial plants emit CH_4 under aerobic conditions it presented quite a conundrum as CH_4 is a highly reduced molecule. The ubiquitous plant structural component pectin is a highly activated methyl donor, and in purified form it emits CH_4 in response to UV-radiation. Therefore, pectin is regarded as one of the most likely precursors in plants to aerobically emitted CH_4. *In vivo*, however, pectin is situated under the cuticle and in between primary cell walls and is very well protected against UV-radiation due to absorption and reflection by the outer epicuticular wax layers. This raises the question to what extent UV-induced CH_4 production may occur at the plant surface wax layer. In this presentation we report the mass spectrometric investigations of the photolytic pathways.

Materials and methods

Seeds of *Brassica oleracea capitata f. alba*, donated by Nordic Gene Bank, were germinated in potted soil and grown for seven weeks in a climate controlled chamber. The *B. oleracea* surface wax was gently removed and exposed to 17 W m^{-2} of UV-B (309-314 nm) for 331 h using Philips PL-S 9 W/01 2 P 1 CT tubes while incubated in gas tight, UV transparent glass vials.

Analysis

Head space aliquots of 500 µl were sampled and identification of the gaseous products (C_1-C_3) was performed with a Varian 3400 gas chromatograph interfaced to a Saturn II ion trap mass spectrometer (GCMS) . The compounds were separated using a PORAPLOT U fused silica column

The leaf surface wax was analyzed by GCMS using a Hewlett Packard HP 6890 gas chromatograph interfaced to a HP5973 mass selective detector. The products were separated using WCOT fused silica column coated with VF-23 and identified using NIST search engine, version of 2.0 f.

UU/VIS spectra were obtained using a HPLC system and a UV6000LP PDA-detector

Results and discussion

A trial resulted in specific CH_4 emission rate of 153 ± 14 nmol CH_4 g^{-1} wax h^{-1} in air when exposed to UV-radiation. The GCMS analysis of the leaf wax revealed ($C_{29}H_{60}$) and 15-nonacosanone ($C_{29}H_{58}O$) as the predominant compounds accompanied by linear C13-C15 aldehydes, 2-pentadecanone and 2-hexadecanone in low concentrations. The surface lipids have only a weak UV absorption tailing into the 300-330 nm region. However, the photolytic products of isolated surface wax as well as the secondary products were identified by GCMS analysis.

In sum, methane formation from leaf surface wax is a two step process initiated by a photolytic rearrangement reaction of the major wax component, i.e. 15-nonacosanone followed by an α-cleavage of the generated ketone. In the latter reaction the presence of concurrent path ways become significant for the yield of methane as only the Norrish I route leads to methane. The ratio of the Norrish I and II routes can be estimated using the two products 1-tridecene (Norrish II) and tetradecane (Norrish I) . This ratio $C_{14}H_{30}/C_{13}H_{26}$ has been estimated to 0,021, and hence the methane route is a minor, but nevertheless significant process.

Mass spectrometry was found to be an excellent analytical tool for rationalization of the photolytic pathways

PTh-059
11:10 – 12:20

Application of HPLC/MS in determination of heroin metabolites in saliva an urine samples

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Keywords:
saliva ; urine ; heroin ; metabolite ; glucuronide

Novel aspects:
Developing accurate, simple, cheap and suitable method in determination of heroin metabolites in saliva an urine with HPLC/MS

Abstract:

Introduction

Urine is a common, and saliva represents an alternative specimen for substances of abuse determination in toxicology. In this study, one step was to optimize a method for saliva and urine specimen preparation for heroin metabolites, codeine, 6-monoacetylmorphine (6-mam) and morphine determination by high performance liquid chromatography-mass spectrometry (HPLC/MS), and then this method was applied on saliva and urine samples taken from the patients. As a preliminary analysis, test strips for opiates identification in patients` urine were used. Saliva and urine samples from patients whose preliminary test was "positive " were taken for further analysis.

Methods

Sample preparation

Both, saliva and urine specimens was prepared using liquid/liquid extraction of codeine, 6-mam and morphineby mixture of chloroform and isopropanol (9 : 1 ; v/v).

HPLCMS analysis

Extracts were analyzed by HPLC-ESI-MS technique : at Waters Alliance ® system, the separation column Waters Spherisorb® 5 μm, ODS2, 4.6 × 100 mm was used ; mobile phase : ammonium acetate : acetonitrile (80 : 20 ; v/v), mobile phase flow rate 0.3 mL/min, autosampler temperature 20° C; injection volume 50 μL; Mass detection range: 100400 m/z, centroid mode, interscan delay 0.1 s, scantime0.5 s, splitless, four voltage values : 70, 60, 50 i 38 V, ES+, source temperature 150° C , desolvation temperature 430° C, gas flow for desolvation 362 L/h and at cone 135 L/h, capillary voltage 3 kV - mass spectrometer Waters Micromass® ZQ™ (Waters Corporation, Milford, MA, USA).

Calibration and optimization were done using morphine standard (ion 286) 10 mg/L at flow rate 10 mL/min. Regression and correlation analyses were performed with the probability level of 0.05. Mass spectra were analyzed by software Waters MassLynx™ (Waters Corporation, Milford, MA, USA).

Results and Discussion

Calibrations for each analyzed substance in both specimens were done in the concentration range from 0.1 to 1 mg/L and the coefficients of correlation were above 0.99. Recoveries for morphine and codeine determination in saliva was 99%, while for 6-mam it was 94 % and recoveries for morphine, codeine and 6-mam for determination in urine were 103 %, 101 % and 93 %, respectively. Limits of detection and quantification of a proposed method were 0.01 mg/L and 0.05 mg/L, respectively for both sample types.

Applying the proposed method on real samples of patients (n=10) where positive reaction was obtained on test strips we determined concentrations of heroin metabolites in selected samples in following ranges : Concentration of codeine in the saliva of the heroin abusers ranged from 0.05 to 5.33, for morphine between 0.05 and 5.33 and for 6-mam between 0.01 and 0.68 mg/L. Concentration of codeine in the urine samples of the same patients ranged from 0.22to 5.74, for morphine between 0.15 and 6.32 and for 6-mam between 0.05 and1.78 mg/L.

A proposed HPLC/MS method for codeine, 6-mam and morphine determination in saliva and urine samples is accurate, simple, cheap and suitable for routine analysis and monitoring of heroin abuse.

PTh-060

13:30 – 14:40

Analysis method of polybrominateddiphenylether using GC-MS and GC-MS/MS coupled with automated identification and quantification system with a database

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Keywords:
GC/MS, GC-MS/MS, polybrominateddiphenylether

Novel aspects:
For analysis of BDEs using GC/MS or GC-MS/MS, the novel method using AIQS-DB and the conventional (isotope dilution method) was developed for easy operation and reduction of standards.

Abstract:
There are 209 isomers of polybrominated diphenyl ether (BDEs) with each one showing different level of toxicity and detection frequency in environmental samples. GC-MS is commonly used for the analysis of BDEs.

A common method of BDEs analysis requires the expensive standards and instrument of gas chromatograph/ double focusing high resolution magnetic sector mass spectrometer (GC-HRMS) . Moreover, the handling and maintenance of GC-HRMS instruments is a time-consuming and labor-intensive work. This situation is critical problem especially for environmental laboratories in under developing countries.

Kadokami *et al.* [1] developed a novel automated identification and semi-quantification system with a database (AIQS-DB) which allows an automatic identification and semi-quantitation of 1,000 pollutants without standard sample analysis. The database includes retention indices, mass spectra, and internal calibration curves for 1,000 pollutants. The pollutants are identified using the mass spectrum and retention time predicted by retention index and retention times of *n*-alkanes. Semi-quantitation is performed using internal calibration curve.

We developed a method for BDEs analysis. For reduction of the necessary authentic standard, AIQS-DB was applied to BDEs with less toxicity and detection frequency, while the conventional method (isotope dilution method) was applied to BDEs with a higher toxicity and detection frequency in order to obtain precise quantitation results. For easy handling and maintenance, single quadrupole mass spectrometer of GC-MS was used.

The developed method was applied to sediment samples. BDEs were detected and semi-quantitation results were obtained. However, several samples with heavy matrix could not detected BDEs due to peak overlapping. GC-MS was not applied to heavy matrix sample analysis such as sediment sample. Gas chromatography triple quadrupole mass spectrometry (GC-MS/MS) was applied to improve the separation of BDEs from heavy matrix, ensuring easy handling and maintenance. Using GC-MS/MS, BDEs were successfully identified and determined, corresponding to the results by the conventional method by HR-GCMS.

1. Novel gas chromatography-mass spectrometry database for automatic identification and quantification of micropollutants, Kiwao Kadokami, Kyoko Tanada, Katsuyuki Taneda, Katsyhiro Nakagawa, *J. Chromatogr A*, 1089 pp 219-226, 2005.

PTh-061
11:10 – 12:20

LC-HRMS investigation of the human in vitro metabolism of brominated flame retardants using APPI and ESI.

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Keywords:
Brominated flame retardants, APPI, High resolution mass spectrometry, metabolism

Novel aspects:
Complementary use of ESI and APPI for complete characterisation of BFRs formed in vitro. HRMS provides discrimination between isobaric hydroxy-polybrominated diphenylethers. New information about metabolic fate of BFRs in human.

Abstract:
Brominated flame retardants (BFRs) are widely used in the production of polymers and plastics in order to prevent fires in electronic and domestic goods. These compounds, mainly represented by tetrabromobisphenol-A (TBBPA) and polybromodiphenyl ethers (PBDEs) , are suspected to act as endocrine disruptors [1,2] and their toxicity is of growing concern. However, although the occurrence of BFRs has been shown in almost all environmental compartments, limited data is available about their fate, in particular in humans.

GC-MS is widely used nowadays for the analysis of PBDEs, though not perfectly suited for the highest MW PBDEs (octa- to deca-BDEs) , mainly due to thermal degradation problems. For TBBPA, a derivatisation step is required. In this work, LC-MS methods based on ESI and APPI associated with high resolution ion analysis, were developed for the analysis of BFRs metabolites, which we expected to be moderately polar to polar molecules. These methods were applied to the study of *in vitro* metabolism of BFRs after incubation with human primary cultures of hepatocytes. HRMS full scan analysis was found to be useful not only for the identification of known metabolites, but also for the detection of non targeted metabolites, which were identified by MS/MS experiments.

First, the efficiency of ESI, APCI and APPI was compared for the analysis of TBBPA, PBDEs and their metabolites formed after incubation with hepatocytes. ESI was found to be well suited for the analysis of TBBPA and their metabolites, as well as for hydroxylated metabolites of PBDEs. We have previously shown that LC-MS using Atmospheric Pressure Photo Ionization (APPI) could constitute a possible alternative for the analysis of PBDEs [3] . In the continuation of our work, the use of APPI was assessed for the analysis of PBDE metabolites with particular emphasis on hydroxylated PBDEs.

After the optimization of APPI ionization parameters (nature and % of dopant, heated nebulizer and transfer capillary temperatures, LC mobile phase composition and flow rate) , a high resolution acquisition was set up on a LTQ-Orbitrap instrument operating in the full scan mode at a resolution of 60,000, which was necessary to get a total resolution of *e.g.* the m/z 498,7 ions formed from both OH-tetra-BDE $[M\text{-}H]^-$ ions and OH-penta-BDE $[M\text{-}H\text{-}HBr]^-$ ions.

The developed methods were then applied to the study of TBBPA and BDE-47 metabolism after *in vitro* incubations with human hepatocytes. Absolute quantification of metabolites was achieved by using radiolabelled molecules. LC-ESI-HRMS allowed the identification of conjugated forms of TBBPA, namely glucuronide, sulphate and doubly conjugated metabolites. For BDE-47, LC-APPI-HRMS allowed not only the identification of expected metabolites such as hydroxylated PBDEs, but also the characterization of other metabolites, such as a tetra-BDE dihydrodiol and conjugated metabolites. For the latter metabolites, identification was completed by the use of ESI, allowing the access to the m/z of quasi-molecular ions.

Results obtained *in vitro* using human primary hepatocyte cultures as well as other human cell lines demonstrate that human cells can biotransform TBBPA into conjugated metabolites and BDE-47 into hydroxylated, dihydrodiol and conjugated metabolites. The potential of these metabolites to be used as biomarkers of exposure in human samples will further be evaluated within the frame of ongoing impregnation studies.

References

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PTh-062
13:30 – 14:40

Information of the behavior of the persistent organic pollutants in the sea around Japan

Masahiro Tsurukawa[1], Motoharu Suzuki[2], Yuki Haga[1], Chisato Matsumura[1], Takeshi Nakano[3], Masayuki Kunugi[4]

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Keywords:
POPs, HCHs, enantioselective analysis, Japan Sea

Novel aspects:
Pollution levels of POPs in sea water and air over the ocean were investigated. Also isomer and enantiomer analysis were conducted to estimate the source and pollutant pathway of POPs.

Abstract:
Although a large amount of Persistent Organic Pollutants (POPs) have been used in Asian countries such as China or Korea, the state of contamination has not been clarified in Japan Sea surrounded by these countries. In this study, pollution levels of POPs in sea water and air over the ocean were investigated by using voluntary sampling of passenger ships. Also isomer and enantiomer analysis were conducted to estimate the source and pollutant pathway of POPs.
The sea water samples (approx. 50L) and air samples (approx.108m^3) were taken by the passenger ship (NYK cruses CO.,LTD , ASKA-2) equipped with concentrating device between 2009 and 2011.Also in downstream site of Chang Jiang, water samples (approx.6L) were taken in 2010 and 2011.
Identification and quantification of POPs were performed using a gas chromatograph, (HP6890N Agilent) /high-resolution mass spectrometer (800D,JEOL Ltd.) equipped with HT-8 PCB capillary column
(60m*0.25mm id. Kanto Chemical) and BGB172 capillary column (30m*0.25mm id, BGB Analytik AG) for enantioselective analysis
In this result, the highest concentration of Hexachlorocyclohexanes (HCHs) was in the sea around the northern Hokkaido, 880pg/L. DDTs was higher in Tsushima straits than in the north of Japan Sea. On the other hand, it is several times higher concentration in Chang Jiang than in around the Sea of Japan, 1300&#65374；2600pg/L (HCH) .
From the view point of isomer pattern, the ratios (α/γ) of Sea waters were apparently different according to the areas. In general, lower α/γ ratios were observed in low-latitude region. Especially in downstream site of Chang Jiang, it was the lowest value,1.4±0.1, and 1.9±0.6 in the seas around Korea,
4.2±0.6 in southwestern Sakhalin. These results seem to reflect the usage of γ-HCH (Lindane) in China and indicate that HCHs was discharged to the marginal seas of the region through rivers in considerable amounts and transported to Japan Sea via oceans.
Dechlorane Plus (DP) is a chlorinated FR additive introduced as a replacement for Dechlorane, or Mirex.
In the past, there are few data of DP in the environment. In this study the concentration of DP in the sea water was investigated for the first time. The range of DP is 0.7&#65374；14pg/L in the sea around Japan and 5 &#65374；16pg/L in Chang Jiang.
As for chiral analyis, it is regarded that enantioenrichment indicates that it was released some time ago and has since been subjected to recycling from water or soil. EF value of α-HCH was close to racemic in southwest Sakhalin (0.5±0.02) ,downstream site of Chang Jiang (0.51±0.02) . In Chang Jiang and the northern Hokkaido, enantiomer fractions (EFs) of alpha-HCH was nearly 0.5 (racemic) . It suggests that those areas were affected by relatively-recent pollutant source.
In Japan Sea, as latitude become higher, EF values tend to become lower. Since 2005's survey , EF values was 0.46±0.05 in middle Japan Sea, 0.44±0.03 in northern Japan Sea (around Hokkaido) ,0.46±0.01 (pacific sideboard of Tohoku area) . These results indicate that southwest Sakhalin, downstream site of Chang Jiang and Tsushima Straits area polluted by relatively new HCHs.
From comprehensive viewpoint, there is a possibility of contamination in Japan Sea, which is caused by the transportation from other countries and polluted area by HCH was found in around Hokkaido.

PTh-063
11:10 – 12:20

LC/ESI(-)-MS/MS analyses of the biotransformation products of dibenzo-p-dioxin by Sphingobium sp. strain KK2

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Keywords:
LC/ESI (-) -MS/MS, dibenzo-p-dioxin, bacteria

Novel aspects:
LC/ESI (-) -MS/MS was utilized to determine biotransformation products of dibenzo-p-dioxin by a bacterium.

Abstract:
Biaryl ether environmental pollutants such as dibenzo-*p*-dioxin, dibenzofuran and their halogenated congeners are considered to be environmentally-persistent pollutants that originate from incineration processes of domestic and industrial waste. There is much interest to know the ultimate environmental fates of these compounds and this includes the contributions of soil microorganisms to their biotransformation. A soil bacterium, *Sphingobium* sp. strain KK22 was recently isolated from a hydrocarbon-degrading microbial consortium and was found to biotransform dibenzo-*p*-dioxin following induction on the 3-ring polycyclic aromatic hydrocarbon phenanthrene. Strain KK22 was exposed to 50 mg/L dibenzo-*p*-dioxin in 50-ml size microcosms and was sampled multiple times over 48 hours. Liquid chromatography electrospray ionization tandem mass spectrometry coupled with UV detection in negative ionization mode (UV-LC/ESI (-) -MS/MS) was conducted by full scan analyses on acidified microcosm organic extracts and revealed multiple putative ions of interest that were not detected in biotic and abiotic controls. Further investigation by CID product ion scanning showed that least two biotransformation products corresponding to the deprotonated molecular ions $[M - H]^-$ = 251 and $[M - H]^-$ = 267 were present. Fragmentation analyses revealed that these compounds appeared to be ring-opened structures derived from initial angular dioxygenation of the dibenzo-*p*-dioxin molecule by strain KK22 and they were tentatively identified as 6-(2-hydroxyphenoxy)-6-oxo-hex-4-enoic acid and a singly-hydroxylated derivative respectively. If confirmed, these chemicals shall represent previously unreported metabolites from dibenzo-*p*-dioxin by a bacterium. Further investigation is continuing to determine the nature of dibenzo-*p*-dioxin transformation by strain KK22 through the utilization of LC/ESI (-) -MS/MS.

PTh-064
13:30 – 14:40

Analysis of Sea-Dumped Chemical Warfare Agents from Sediment Samples Taken at in Baltic Sea

Ullastiina Hakala, Martin Söderström, Olli Kostiainen, Jenni Taipalus, Paula Vanninen

VERIFIN/Department of Chemistry/University of Helsinki, Finland

Keywords:
chemical warfare agents, Baltic sea, environmental analysis, GC-EI/MS, LC-APCI/MS/MS

Novel aspects:
Novel information of CWA contamination of the Baltic sea.

Abstract:
After the Second World War, a large amount of chemical warfare agents (CWAs) were dumped at the Baltic Sea. For example, in the Bornholm dumpsite located within Danish economic zone, it is estimated that over 500,000 shells and containers containing over 11,000 tons of chemical warfare agents were dumped in 1947 by the Soviet Military Authority in Germany.

In this study, sediment samples were collected during different sampling cruises conducted in the Bornholm dumpsite or around it between 2006 and 2011. The target chemicals were mustard, tabun, Clark I & II, Adamsite, Lewisite I & II and α-chloroacetophenone as well as arsenic oil containing Clark I, triphenylarsine, phenyldichloroarsine and trichloroarsine, and related degradation products. After an appropriate sample preparation procedure, the samples were analysed using GC-EI/MS with selected ion monitoring (SIM) as such and after derivatisation with propanethiol, and LC-APCI/MS/MS with selected reaction monitoring (SRM) as such and after oxidation using hydrogen peroxide.
Analysed sediment samples showed considerable spreading of arsenic-containing chemicals within the dumpsite as well as between the dumpsite and the Bornholm Island. The highest found sediment concentrations have been over 16,000 ng/g of degradation products of Clark I and 39,000 ng/g of triphenylarsine within the primary dumpsite. [1]

Baltic Sea contains also unexplored areas, e.g. Gotland and Gdansk deep, that have been claimed as dumping sites of CWAs. The Chemical Munitions Search and Assessment (CHEMSEA) project has been established to carry out investigation at the official and unofficial dumping areas in the Gotland and Gdansk deep. The main focus of the project is to locate the dumped CWAs and sample the surrounding environment to assess the possible threat. The outcomes of the project will also include recommendations on operating procedures, and guidelines to employ in those areas, to be used by maritime administrations of respective countries, as well as a contingency plan for risk management. The CHEMSEA project is a transnational collaboration including project partners from five EU countries and a number of associated organizations, including governmental agencies and international organizations. This project is part of the Baltic Sea Region Program and is partly financed by the European Union. The project was started in the fall of 2011 and will continue until early 2014. [2]

References

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PTh-065
11:10 – 12:20

Online SPE LC-MSMS for screening and quantifying anti-cancer drugs and metabolites in hospital's wastewaters and rivers

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Keywords:
cancer drugs, waste water, LC-MSMS

Novel aspects:
The main difficulty is the wide panel of chemical structures to be separated using a single rapid automated method

Abstract:

Introduction :
Over 50 cytotoxic chemotherapies are used in hospitals. The main anti-cancer drugs used in cancer chemotherapy can be classified into several categories : cytotoxic, the most represented, but also hormones, immune response modifiers and antibodies. Most cytotoxic agents used in cancer chemotherapy interact with DNA or its precursors. Very few studies are evaluating the future of these drugs in wastewater. Potential risks associated with these discharges are poorly understood and require study work and research to better understand the hazards, exposure characterization and assessment risks to human health and the environment. The purpose of the study is to establish an analytical methodology to screen most of the anti-cancer drugs currently used in hospital waste waters.

Methods :
The goal was to develop a simultaneous analysis of a large number of anticancer drugs from water samples with a limited amount of sample. These samples are processed by on-line solid phase extraction (SPE) to isolate and concentrate the different cytotoxics. Following extraction, compounds are transferred to an UHPLC column for separation. Detection is performed using Multiple Reaction Monitoring mode on an ultrafast triple quadrupole mass spectrometer. Special attention was given to the orthogonal selectivity and to the working pressure compatibility of the extraction and analytical columns.

Preliminary results:
The UHPLC system (NEXERA, Shimadzu Corp.) is equipped with an online SPE cartridge which, thanks to the innovative switching valves configuration, is isolated from the high pressure LC. This allowed us to combine on-line SPE with UHPLC. The first step of the project was to optimize the MRM transitions for each molecule of the chosen list and to choose the best mobile phase composition compromise for optimum sensitivity. Once that process done for the 25 selected drugs, several SPE sorbent for extraction of a wide range of different chemical class compounds were tested. The same study was conducted on the choice of the analytical column trying to combine several retention mechanisms. The final stage of development of the method was devoted to optimization of the sensitivity to reach the lowest limit of quantification and to study of the dynamic range for each molecule. Finally the methodology was validated with real wastewater sample to assess extraction and ionization recovery as well as ruggedness of the system.

PTh-066
13:30 – 14:40

Identification of chlorinated aromatics as impurity of chlorinated paraffins by GC-HRMS or GC-HR-Tof-MS

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Keywords:
POPs, Chlorinated Paraffins, Impurities, Identification, PCB

Novel aspects:
Identified high levels of POPs in technical CPs and products by GC-HRMS or GC-HR-Tof-MS

Abstract:
Chlorinated paraffins (CPs) are industrial products used as metal-working fluids and flame retardants for plastic materials. Short chain CPs (SCCPs) seems to persist in the environment and bioaccumulate in biota, and are under review by the Stockholm Convention on persistent organic pollutants.
We identified high levels of POPs such as PCBs, PCBz, PCNs and HCHs from air sampler materials. After detailed investigations these POPs are concluded as impurities of technical chlorinated paraffins (CPs) , which used for rubber materials as flame retardant at 2 - 6 % contents. The exact mass spectrum and accurate mass of these chemicals are fixed theoretical mass with few ppm errors. The profile of PCBs congeners are close to technical PCBs, which suggest chlorination of biphenyls as impurities of paraffins. The homologue profiles of PCBs are relatively lower chlorination pattern with some specific congeners.
These technical CPs are imported from China. The production of total CPs in China has continued to increase, reaching 600,000 tones in 2007. Although the huge production and use of CPs in China could imply potential contamination of various media, there is little information on exposure to SCCPs. In our previous study, the analytical methods for SCCP were investigated in detail and applied for dietary samples. Preliminary evidence on the significant increase of SCCP in food sample Beijing in 2009 warrants urgent investigations to refine dietary intake estimates by targeting food types and source identification.
On the other hand, the finding high levels of legacy POPs as impurities in technical CPs are more serious for POPs inventories around the world.

PTh-068
13:30 – 14:40

Determination of absolute configuration of PCB atropisomer and analysis of enantiomeric excess in the human sample

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Keywords:
PCB atropisomer, human sample, circular dichroism, RI-CC 2 /TZVPP calculations, enantiomeric excess

Novel aspects:
Absolute configuration of PCB-183 (aS and aR) were determined, aS-PCB-183 and aR-PCB-183 in serum are determined enantio-selectively.

Abstract:
The electronic circular dichroism (CD) spectra of a pair of enantiomeric 2,2',3,4,4',5',6-heptachlorobiphenyls (PCB-183) were investigated for the first time experimentally and theoretically. Geometrical optimization at the DFT-D 3 -B-LYP/TZVP level revealed that the two phenyl planes of PCB-183 are nearly orthogonal (89°) . Due to the sterically large chlorine atoms, PCB-183 becomes atropisomer. Thus, optical resolution was performed by chiral HPLC (column : OJ-H) , affording enantiomerically pure first elute (100% ee) and optically enriched second elute (74% ee) . Their experimental UV-vis and CD spectra in n-hexane were compared with those obtained by theoretical calculations at the RI-CC 2 /aug-TZVPP level.
Determination of absolute configuration is crucial in the structure elucidation and their biological activities of chiral compounds, and it is also true for PCB atropisomer. However, the determination of absolute configuration of PCB has never been performed. Here absolute configuration of PCB-183 (aS and aR) were determined, aS-PCB-183 and aR-PCB-183 in human sample are determined enantio-selectively.
Sample collection : The serum and adipose tissue samples were obtained after receipt of written informed consent. After about 10ml of blood were centrifuged by 3000 rpm, serum samples were obtained and stored in -20° C until analysis.
Extraction and cleanup : About 2 g of serum was added cleanup spike solution, 2.5ml of diethyl ether, and 5 ml of ethanol, then extracted with 10ml of hexane twice. Hexane extracts were passed through 1 g of florisil/ 1 g of silica gel double layer column (Supelco Inc., U.S.) , eluted with 15ml of 15 % diethyl ether/hexane. Eluate was concentrated to 0.1ml under gentle stream of nitrogen, and then added syringe spike solution[1)].

GC and LC conditions for chiral separation
To determine absolute configuration of each PCB atropisomers, the enantioselective separation of PCB atropisomer was performed using HPLC (TOSOH CO-8020, SHIMADZU LC-10AT) with CHIRALCEL (DAICEL) OJ-H (4.6mmIDx150mm) . The adsorbent is cellulose tris (4 -methylbenzoate) silica gel coating type, particle size 5 μm, sample loop : 20 μL, column temp : 38° C, n-hexane was used as elution solvent with flow rate 0.3mL/min, UV 291nm.

PCB-183 in human sample was enantio-selectively determined by GC/HRMS. (JEOL JMS-800D) using BGB-172 column (20 % *tert*-butyldimethylsilylated *beta*-cyclodextrinin methylphenylcyanopropylpolysiloxane, 30m length x 0.25mmID, 0.25 μm Film Thickness, BGB Analytik AG) . Carrier gas was helium, and injector and transfer line temperature were 230° C and 245° C. 1 μl of samples were injected splitless at an initial temperature of 120° C, 4 ° C/min to 180° C, 1 ° C/min to 230° C, and held for 10minutes. The ion source was operated in the electron-impact mode (EI, 38eV, 250° C) .

Experimental data and theoretical calculation results
The concentrations is calculated as epsilon (209) =73000. Experimental CD spectra of aR/M are roughly similar in comparison with theoretical calculation of aS/P. (aS : axial S) . In experimental CD spectra, comparison between fraction A (100%ee) and fraction B (74%ee) multiply 1.35 with inversion of sign were in excellent agreement. The first elution peak on BGB-172 column by GC/MS is assigned as aS/P. The first elution peak on OJ-H column by LC/MS was also assigned as aS/P.

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PTh-069 Analysis of natural organic dyestuffs extracted from textiles.

11:10 – 12:20

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Keywords:
masspec, dyestuffs, natural, historical, textiles

Novel aspects:
During the first phase of colour fading of textiles the aglycone concentration is increasing because of the O-glycosidic bond breakdown.The moment O-glycosidic bonds are completely hydrolysed, aglycone concentrations begin sinking.

Abstract:
Study of the fading of natural dyes in (historical) textiles is rather complicated because of low concentration of investigated chromophores plus a big surplus of matrice elements (textile fibres and degradation products) and because of the lack of significant portions of the testing material.
Most suitable method is extraction/soft hydrolysis by mixture of methanol formic acid (95 : 5) followed by LC-MS analysis. Most of the previous papers were concentrated on the analysis of chromophores themselves (aglycones) while glycosylated chromophores were not the principal target. The main reason was that the extraction by mixture of 37% HCl/MeOH/H 2 O (2 : 1 : 1) would hydrolyse O-glycosylated chromophores. Therefore, it is not possible to study the concentration changes of O-glycosides along with their artificial aging.

The overall goal of this work is to devise efficient techniques for extracting dyes from textile samples without simultaneously decomposing some of the dye components. Moreover, this type of extraction is more suitable for MS-analyzers, as HCl is a corrosion agent and, at the same time, formic acid can be readily evaporized after the extraction.
Silk textiles were dyed by mordant dyes (anthraquinones) extracted from the madder roots and exposed to two types of artificial aging (thermal and photooxidative) , before and after the aging experiment. Analyses of the samples were carried out by extraction, 30 minutes-sonification at 60 ° C followed by evaporation of the solvents in a N_2-stream. Separation of analytes was carried out by gradient reverse phase chromatography on C18-column. Mobile phases were
water and/or acetonitrile both with 0,1 % of formic acid. ESI in negative mode and HRMS detection using ORBITRAP technology were used for the analysis. Monitoring analytes were rubyretric acid (alizarin primeveroside) , alizarin glucoside, alizarin, lucidin primeveroside, lucidin glucoside, lucidin, galiosin (pseudopurpurin proveroside) , rubiadin primeveroside, rubiadin, purpurin.

Conclusions
During the first phase of colour fading of the analysed textiles the concentration of the aglycone itself (not bonded to the sugar moiety) is increasing because of the O-glycosidic bond breakdown (which is the most vulnerable to a fission) . The moment all O-glycosidic bonds are completely hydrolysed, aglycone concentrations begin to sink. O-primeverosides first change onto O-glykosides of aglycones. The fotooxidation is more destructive then thermal conditions.
The most stable are O-glycosides and O-primeverosides of alizarin, which at the same time are the most concentrated chromophores in madder. Lucidin, rubiadin and pseudopurpurin are more degraded than alizarin. These facts lead to search first in historical textile for aglycons of chromophores, these are more stable.
The single MS spectra of glycosylated aglycones are always accompanied by typical daugther ions of the aglycones $[M\text{-}162{,}053]^-$ for O-glucosides and $[M\text{-}294{,}095]^-$ for O-primeverosides occuring in the ESI ionizator.

PTh-070
13:30 – 14:40

Atmospheric pressure ionisation and ion mobility novel techniques for the analysis of the POPs on the Stockholm convention including dioxins

Bert van Bavel[1], Jody Dunstan[2], Peter Hancock[2], Rhys Jones[2], Ingrid Ericson[1], Jessika Hagberg[1]

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Keywords:
APGC, ion mobility, POPs, dioxins, Stockholm Convention

Novel aspects:
First time data on APGC and dioxins and ion mobility

Abstract:
Within the UNEP program '*Assessment of Existing Capacity and Capacity Building Needs to Analyze POPs in Developing Countries*' several activities were undertaken during the period from 2006 to 2010. The program is focused on the development of analytical capacity for the POPs under the Stockholm Convention including several pesticides (DDT, chlordane, toxaphene) and industrial (by) -products (dioxins, PCBs) . Recently brominated flame retardants (BDE) and an organic fluor compound (PFOS) were added to the convention. One of the conclusions of the program was that it is quite a challenge to analyze all POPs in the sample types proposed for the global monitoring program (GMP) . This is especially through for countries in developing countries.

Traditionally low resolution GC/MS is used for the analysis of the chlorinated POPs, mostly operating in the EI mode or NCI mode for specific compounds including toxaphene and the brominated BDEs. For dioxins high resolution GC/MS systems are often required to avoid inferences and to achieve the low LODs needed. One of the difficulties to develop a universal method for the Stockholm convention POPs is that different ionization techniques are needed for different POPs.

Preliminary results show that APGC might be a way forward to the development of a universal detection system based on mass spectrometry. Atmospheric pressure ionisation under charge transfer conditions often results in very soft ionisation compared to EI or even NCI conditions. For many of the POPs on the Stockholm convention only the molecular ion is formed under these conditions. By avoiding fragmentation the sensitivity in the MRM or SIR mode is enhanced. Especially for compounds known to show extensive fragmentation (for example Aldrin, Dieldrin) under EI conditions, the results using APGC showed nearly exclusively the molecular ion. Sensitivity was tested for dioxins using the APGC ion source with dry N_2 on a Waters Xevo TQ-S in the MRM mode. The results were surprising and the sensitivity was comparable with high resolution GC/MS, the standard for dioxin analysis. Injection of a 1/10 dilution of calibration standard CSL (Wellington Labs, Ontario, Canada) of concentration range 10-100 fg/ul resulted in S/N from 10 to 65.

Another problem of the isomer specific dioxin analysis is the co-elution of non-toxic PCDD/DF congeners with the 17 toxic isomers to be analysed according to the TEQ concept. Currently no commercial column available is able to separate all congeners, and confirmation analysis has to be performed on a different GC column. Ion mobility might be able to a solution to this problem, by separating 'critical' PCDD or PCDF congeners in an ion mobility cell. Normally only used for larger (bio) molecules recent developments have also shown a potential to separate smaller molecules, thus adding an additional MS separation dimension. Preliminary results using ion mobility to separate 'critical' dioxin and furans pairs will be presented.

PTh-071
11:10 – 12:20

Mixed-mode Liquid Chromatography/Tandem Mass Spectrometry Analysis of Glyphosate and Its Application in Exposure Study

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Keywords:
glyphosate herbicide, LC/MS-MS, mixed-mode liquid chromatography, multiple reaction monitoring (MRM) , direct aqueous injection (DAI)

Novel aspects:
A direct aqueous injection mixed-mode liquid chromatography/tandem mass spectrometry method was developed and applied to a large scale whole ecosystem glyphosate exposure experiment for the first time.

Abstract:
Glyphosate-based herbicides are the most commonly used herbicides in Canadian agriculture and silviculture. However, it is quite challenging to monitor glyphosate and its related compounds in the environment due to their high polarity, high water solubility, low volatility, and the lack of chromophore and fluorophore in the molecules. Tedious and time-consuming derivatization is often required for the analysis. Taking advantage of the multimode (reversed-phase and weak anion-exchange combined) separation mechanism offered by the Acclaim WAX-1 column, a direct aqueous injection (DAI) mixed-mode liquid chromatography/tandem mass spectrometry (LC/MS-MS) method was developed for the determination of glyphosate, aminomethylphosphonic acid (AMPA) and glufosinate. The analysis was carried out by using an electrospray ionization (ESI) source in negative ionization and multiple reaction monitoring (MRM) scan mode. Two MRM channels were monitored for each target compound to achieve positive identification. Aqueous environmental samples were directly injected and analyzed in 12 minutes with no sample concentration or derivatization steps. Isotope labelled internal standards ^{13}C, ^{15}N-glyphosate and ^{13}C, ^{15}N-AMPA were used to compensate matrix effects during the measurement. The instrument detection limits (IDLs) for glyphosate, AMPA and glufosinate were 1, 2 and 0.9 μg/L, respectively.

The method was applied to determine glyphosate residue concentrations for a large scale whole ecosystem experiment designed to access potential ecotoxicological effects of glyphosate based herbicides in small wetland systems. Each naturalized small wetland was split in half with impermeable plastic barriers so that one side of the wetland would be treated with formulated glyphosate herbicides (VisionMax or Roundup WeatherMax) and the other side would serve as untreated control. The herbicide formulation was applied at different rates to yield two different aqueous exposure concentrations, one was the relevant level in either agriculture environment (210 μg a.e./L) or forestry environment (550 μg a.e./L) and the other was the predicted worst case scenario exposure level (2,880 μg a.e./L) . Herbicide treatments were applied to wetlands in replicates ($N = 5$ or 6) either alone or in combination with nutrient additions. The concentrations of glyphosate at specific periods after treatments were monitored by the LC/MS-MS method for both the treated side and the control side of each wetland. Selected samples were also analyzed by another gas chromatography method using nitrogen-phosphorous detection (GC-NPD) after derivativzation of glyphosate and AMPA for comparison purpose. The results demonstrated that the novel mixed-mode LC/MS-MS method was effective and reliable for the analysis of glyphosate based commercial herbicide formulations in various surface water samples. This method would provide a quick, easy and accurate approach for the determination of glyphosate and its related compounds in the environment to satisfy the needs for emergency response, water quality monitoring and regulation enforcement.

PTh-072
13:30 – 14:40

Desorption atmospheric pressure photoionization-mass spectrometry for the determination of chemical composition of atmospheric aerosols

Evgeny Parshintsev[1], Ingrid Lamazere[1,2], Jose Ruiz-Jimenez[1], Tiina Kauppila[1], Kari Hartonen[1], Tapio Kotiaho[1], Marja-Liisa Riekkola[1]

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Keywords:
DAPPI-MS, atmospheric aerosols, filter samples, organic compounds, semiquantification

Novel aspects:
DAPPI-MS was applied for the first time to ambient atmospheric aerosol samples with possibility of semiquantification using tandem mass spectrometry

Abstract:
The Earth's climate is a highly dynamic and complex system in which aerosols have been increasingly recognized to play a key role. Because of the complexity, quantification of the climate effects of aerosols is still uncertain and is a challenging aspect in climate research. Qualitative and quantitative information about the compounds in aerosol particles is essential if we are to comprehend their role and effects in the atmosphere. To date, numerous analytical techniques, both on-line and off-line, have been developed in order to determine size-dependent chemical composition of ambient aerosol particles[1,2]. They all, however, have different disadvantages, including complicated sample preparation, which may alter the original chemical composition, low time resolution or, e.g. in aerosol mass spectrometry, mixed spectrum of many compounds present in the particle which is difficult to interpret. Desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS)[3] offers an alternative to the existing on-line techniques. DAPPI-MS has been developed for the mass spectrometric analysis of polar and non-polar compounds directly from a variety of surfaces. In DAPPI, the analytes are desorbed from the sample surface using heated solvent flow, after which the gaseous analytes are ionized via photon initiated gas-phase reactions. In this research, semi-on-line analysis, based on filter sampling of all-size ambient aerosols, and DAPPI-MS with consecutive DAPPI-MS[2] was developed. This approach allows the verification of structures of compounds found in aerosols by tandem mass spectrometry. The DAPPI-MS system gave linear response over the calibration range from 20 to 400 ng applied on filter for over 30 studied compounds (acids, aldehydes and amines) . This allowed semiquantitative analysis of the compounds from the filter samples. Since no sample preparation was required and since the sampling system can be easily integrated to DAPPI-MS analysis, the method developed herein can be considered highly promising in the field of atmospheric research.

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PTh-073

11:10 – 12:20

Evaluating multiplex fragmentation and ion mobility separations to improve the quality of rapid LCMS peptide mapping analyses for biotherapeutic proteins.

KATSUTOSHI NAGASE[1], KENJI HIROSE[1], Scott Berger[2], Henry Shion[2], St John Skilton[2], Weibin Chen[2]

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Keywords:

Ion mobility, peptide mapping, biotherapeutic

Novel aspects:

Ion mobility gas phase separations and multiplexed fragmentation acquisition improve peptide assignment quality for rapid LCMS peptide mapping of biotheraputics.

Abstract:

Introduction

LCMS Peptide mapping is a fundamental technique for defining the primary structure of biotherapeutic proteins. Improvements in separations, mass detection, and informatics have reduced acquisitions to an hour, and data processing from days to hours. Such assays are invaluable for biotherapeutic characterization and identification of product variants, but lack the throughput required to become effective screening tools supporting several early (clone screening, QbD) and late (Formulations, Stability) development activities. Modern TOF analyzers have enabled the collection of rapid accurate mass peptide mapping data, but accurate mass identifications are ambiguous for some peptide assignments, and constitute insufficient evidence for confident identification of new variants. Here, we investigate how multiplexed fragmentation and ion mobility LCMS can provide more confident, rapid map assignments.

Methods

Analytical-scale UPLC-QTof MS methods were developed for rapid (5, 10 min gradient) , and typical (90 min) length peptide maps of a biotherapeutic IgG 1 monoclonal antibody. Accurate mass precursor and multiplexed fragmentation data were acquired using LCMSE acquisition methodology. For conventional 90 min acquisitions, the precursor/ fragmentation (MS/MSE) duty cycle was 1 sec, equally divided, reduced to 0.4 sec cycle time during rapid analyses. This enabled sufficient data points across ion peaks to facilitate precursor identification and quantification, and establish chromatographic linkage of precursors and their fragments. In selected experiments, the integral ion mobility functionality was enabled, permitting additional ion separation prior to the collision cell, with other parameters unchanged. Data processing was accomplished with a developmental version of BiopharmaLynx software.

Preliminary Data

Initial results indicate that rapid monoclonal antibody peptide maps using 10 minute gradients are capable of yielding high coverage (>90 %) peptide maps with readily interpretable MSE multiplex fragmentation patterns that can be used to validate accurate mass assignment of peptides. Multiple cases of peptide coelution (peptides with chromatographic profiles having apex elution within 1 / 7 th of a chromatographic peak width at half height) were observable, resulting in the generation of chimeric multiplex fragmentation spectra. This chimeracy does not preclude using such ions in a confirmatory role (e.g. a quality metric of minimum number or percentage of accurate mass b/y ions to validate an accurate precursor mass assignment) . Parallel studies conducted with the ion mobility functionality enabled showed the elimination of fragment ion chimeracy, as the resulting data processing required not only that MSE fragment ions share chromatographic retention profiles with their precursor ions, but also that they exhibit common ion mobility drift time with those precursors. The 10 min gradient studies indicated that Ion mobility improved data clarity, but did not appear to be essential for fragment ion validation of accurate mass assignments. Therefore, we have undertaken a second round of antibody peptide map analyses using an even shorter 5 min gradient elution. Results of the shorter experiment indicate that coverage was not negatively affected by the shorter gradient, and that coelution/chimeracy issues were more prominent. We are currently evaluating the results of the ion mobility enabled analyses at this shorter gradient length. Further work is ongoing with both rapid peptide mapping workflows applied to additional biotherapeutics to factor in map complexity to these studies, and assess the dynamic range and quantitative reproducibility of the delivered results.

PTh-074
13:30 – 14:40

Conformational and functional analysis for quality control of protein drugs using ion mobility mass spectrometry

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Keywords:
antibody, conformation analysis

Novel aspects:
Protein structural differences in the liquid phase can translate into measurable conformational differences apparent in the gas phase mobility of protein ions.

Abstract:
Background : A Conformational change of biopharmaceuticals affects pharmacological potency and property of final drug product. A new category of mass spectrometer has been developed with capability to fractionate biomolecules in gas phase by size, shape, and charge (via an ion mobility separation) prior to MS detection. Ionmobility mass spectrometry (IM-MS) , which has the capability to fractionate molecules in a gas phase by size, shape, and charge prior to MS detection, is a useful device to predict the conformational change of biomacromolecule. This capability can be exploited by researchers to simplify analysis of complex samples, or profile structural hetetogeneities within a protein preparation. This study can show direct application to the analysis of protein structural heterogeneity and will provide the strategy of quality analysis of biopharmaceutics.
Methods : A Synapt HDMS system (Waters) was operated in mobility-TOF mode for all analysis. Mass Lynx was used for instrument control and data processing. Trastuzumab (Herceptin) and Eternercept (Enbrel) were used as model biopharmaceuticals and prepared in phosphate buffer to the right consistency. Etanercept in phosphate buffered saline, pH 7.2, was heated at 95 degree from 2 min and 10 min and afterward cooled to 4 degree. Trastuzumab was gradually heated from 60 to 90 degree over a period of 15 min and then cooled to 4 degree. The bioactivity of Trastuzumab was evaluated with bioassay using BT-474 cells. BT-474 cells in exponential growth were seeded on day 1 in 96-well plates at 1 x 10^4 cells per well. They were allowed to adhere over night and then treated with non- or heat-treated Trastuzumab at indicated doses. After 5 days of incubation, WST-8 proliferation assay was performed as supplier protocol.
Results : A different mobility shift was detected between the denatured antibody drug and the non-treated sample by IM-MS analysis. IM-MS data processing involved the generation of three-dimensional m/z versus drift time versus intensity plot scalled Driftgram. Driftgrams of each different denatured condition were different in each samples. Significant differences can be observed by comparing the ion mobility data for the 28+ charge state, which is the strongest signal in driftgram. In addition, the extent of this shift depended on the denaturating conditions. Furthermore, the bioactivity of Trastuzumab was correlated with the degree of change in denaturating conditions. The control sample was shown the cytotoxicity to this cell line, but heat-denatured samples were decreased the cytotoxicity. While we have to perform further investigation, conformational study using IM-MS might be useful to evaluate the quality check of protein drugs.
Conclusions : Ion mobility separations provide an additional separation dimension that permits resolution of underlying protein structural distributions within individual charges states of a protein electrospray mass spectrum. Protein structural differences in the liquid phase can translate into measurable conformational differences apparent in the gas phase mobility of protein ions. Scientists can use this capability to study the stability, refolding, and underlying structural heterogeneity of biotherapeutic proteins. Such structural experiments can be accomplished by MS infusion studies requiring only minutes of data acquisition.

PTh-075
11:10 – 12:20

Effects of hydrophobic oligopeptides on the aggregation process of Parkinson`s disease target protein alpha-Synuclein

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Keywords:
Parkinson`s disease, alpha-Synuclein, oligomerization,

Novel aspects:
Based on the sequence of the central amyloidogenic domain hydrophobic oligopeptides were used in order to accelerate or prevent the aggregation process of the Parkinson`s disease key protein alpha-Synuclein.

Abstract:
Synopsis

The intrinsically disordered protein alpha-Synuclein (αSyn) is known to be implicated in both idiopathic and inherited forms of Parkinson´s disease (PD). The pathological hallmark of sporadic PD is the formation of intracyoplasmic fibrillar deposits in dopaminergic neurons (Lewy bodies) in the substantia nigra. The amyloid fibrils are formed by conformational changes from α-helices to β-sheets. Accordingly, one strategy to overcome the disease would be to find a drug that could stabilize the α-helical conformations and/or destabilize the β-sheet conformations of the amyloidogenic protein[1]. The 140 amino-acid αSyn consists of three main domains: (i), an N-terminal region (1-60) containing several imperfect KTKEGV repeats; (ii), a hydrophobic non-amyloid component of Alzheimer`s disease (NAC) region (61-95); and (iii), a highly negatively charged C-terminal region (96-140). The hydrophobic NAC domain has a critical role for aggregation, especially the hydrophobic amino acid stretch Val71-Val82. However, how αSyn is involved in the pathogenesis of neurodegenerative disease is not understood at present. Here we report the influence of synthetic hydrophobic oligopeptides, so called β-sheet breaking peptides, on the aggregation process of the Parkinson`s key protein αSyn.

Methods

Recombinant expressions of full-length αSyn and αSyn polypeptides were performed using the *E. coli* expression system BL 21 (DE 3) [pLys] strain and the T7 RNA polymerase system. Six different hydrophobic oligopeptides were manually synthesized according to Fmoc-strategy on TGR-PHB resins. HPLC purification of αSyn and αSyn peptides were performed with Thermo-3000 semi preparative system on a Vydac-C4 column (250x4.6 mm) with a linear gradient (0.1 % trifluoroacetic acid with 0-80 % acetonitrile) at a flow rate of 1 mL/min and the purity was verified by ESI-MS.
Purified αSyn wt, βSyn, oligopeptides or mixture of αSyn or βSyn and oligopeptides were dissolved in 20 mM Na_2HPO_4 pH 7.5 at concentration of 30 µM and incubated at 37° C with agitation for several days. Electrophoretic separations were performed by Tris-Tricine PAGE with 12-15% on a Mini Protean-3 cell and stained with Coomassie brilliant blue and/or silver nitrate.

Preliminary data

Application of IMS-MS to oligomerization-aggregation mixtures of αSyn *in vitro* recently provided the first identification of specific autoproteolytic truncation and degradation products. In particular, a highly aggregating fragment was identified by cleavage between Val71 and Thr72 in the central aggregation domain of αSyn[2].
The *in vitro* oligomerization of αSyn was investigated by incubation at 37° C in sodium phosphate buffer (pH 7.5) for up to 25 days. The formation of oligomers and αSyn species with molecular weights lower than that of full-length αSyn were observed with slowly increasing abundances over two weeks. Comparative *in vitro* studies of the non-aggregating brain protein, beta-Synuclein (βSyn), which lacks the central amyloidogenic domain (residues 72-83), showed neither oligomerization-aggregation nor any autoproteolytic cleavage within 21 days of incubation. Focused on the sequence of the central amyloidogenic domain and in consideration of the fact that proline preclude β-sheet structure we synthesized six hydrophobic oligopeptides in order to accelerate or prevent the aggregation process of the Parkinson`s key protein αSyn. The interactions were studied by gel electrophoretic separation, immunoblotting, circulardichroism and IMS-MS.

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PTh-076
13:30 – 14:40

Analysis of different conformers of carbonic anhydrase2 using ion mobility coupled with electrospray ionization

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Keywords:
ion-mobility-spectrometry, conformation analysis, ESI

Novel aspects:
The presence of five different discrete conformers of CA 2 was confirmed by using ion mobility coupled with electrospray ionization.

Abstract:
[Introduction]
Ion mobility spectrometry (IMS) coupled with electrospray ionization (ESI) has been recognized as a useful method for the analysis of conformation of proteins, because ESI generates gas-phase protein ions reflecting the conformations in solution and IMS separates gas-phase protein ions according to different collision cross sections corresponding to shape or conformations even at the same m/z values. Here we analyzed IMS driftgrams for a distribution of charge states of the gas-phase carbonic anhydrase 2 (CA 2) ions produced from ESI processes. The driftgram obtained suggested the presence of several different stable conformations of CA 2 ions. The conformations of CA 2 in gas-phase and liquid-phase have been discussed on the basis of the ESI processes such as 1. charge equilibrium state of CA 2 in buffer solution, 2. charge separation in high electric field, 3. charged droplet formation, 4. desolvation, and 5. adiabatic expansion in orifice region.

[Method]
Mass spectra were acquired on a SYNAPT HDMS quadrupole IMS orthogonal acceleration TOF mass spectrometer and MassLynx data processor (Waters Corp., Milford, MA). A protein solution prepared at pH3.6 was infused into the ESI source at a flow rate of 20 microL/min. An electrocapillary voltage and sampling cone voltage were 2.8 kV and 30 V, respectively. A source temperature and desolvation temperature were 100°C and 200°C, respectively. For ion mobility measurement, nitrogen was used as a buffer gas, and IMS cell pressure was maintained at 0.5 mbar. The IMS wave velocity was 300 m/s and the wave pulse height was 7.9 V. CA 2 was purchased from Sigma (St. Louis, MO).
[Results and discussion]
The positive ion ESI mass spectrum of CA 2 obtained from the solution at a relatively acidic condition of pH3.6 showed a distribution of charge states 8+ to 32+, which has a bimodal pattern constituting of 11+ and 17+ corresponding to local maximum peaks and a local minimum peak of 15+. The distribution pattern indicating a conformational state of CA 2 suggested the presence of both extended and folded conformations in solution. At pH3.6, the resulting highest charge state of 32+ was consistence of the number of basic amino acid residues (9Arg, 16Lys, 11His), so that the conformations of CA 2 ion are determined by the combination of protonation on the basic residues. To obtain the information about gas-phase conformations of CA 2 ions, the IMS experiments were performed upon all the charge state CA 2 ions from m/z 908.1 (32+) to m/z 3629.4 (8+). The resulting drift time distributions from the charge states 32+ to 26+ showed singlet patterns suggesting the presence of single conformation **I** of CA 2 ion. The peak corresponding to the conformation **I** was observed from 32+ to 17+. The drift time distributions from 25+ to 20+ showed doublet patterns suggesting the presence of different two conformations **I** and **II**. The peak corresponding to the conformation **II** was observed from 25+ to 14+. The drift time distributions for 18+ and 17+ showed quartet patterns suggesting the presence of four different conformations **I**, **II**, **III** and **IV**. The conformations, **III** and **IV** were observed from 19+ to 13+, and 18+ to 13+, respectively. The drift time distributions for 12+ and 11+ did not show any splitting patterns but broaden peaks, while those for 10+ and 8+ showed singlet sharp peaks suggesting the presence of a folded native conformation **V** of CA 2 ion.

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PTh-077
11:10 – 12:20

The Accuracy and Reproducibility of Calibration in Trapped Ion Mobility Spectrometry

Victor Fursey, Mark Ridgeway, Desmond Kaplan, Melvin A Park

Bruker Daltonics, Billerica, MA, USA

Keywords:
Trapped Ion Mobility Spectrometry, Calibration stability, Calibration accuracy, Comparison with conventional IMS

Novel aspects:
First quantification of TIMS accuracy and stability for providing data directly comparable to conventional IMS with nitrogen drift gas.

Abstract:

Introduction

Trapped Ion Mobility Spectrometry (TIMS) is a recent addition in the field of mobility selective analyzers. In TIMS, ions are radially confined in a quadrupolar ion funnel by RF voltages while axially trapped by the balance of the frictional force in a gas flow and the electric force generated by a DC electric field (V_{tunnel}) that varies as a function of position within the TIMS device. Reducing the DC electric field strength over time allows trapped ions to sequentially elute from low to high mobilities. By calibrating a list of known reduced mobility (K_0) values vs. $1/V_{tunnel}$, K_0 can be determined from V_{tunnel} and compared directly with results from conventional IMS systems.

Methods

A list of compounds varying in mass, charge, and compound class with known K_0 values was compiled from available literature as well as experimentally determined K_0 values acquired using a custom built two meter conventional IMS system with nitrogen drift gas. These compounds where then analyzed using a research prototype TIMS-Q-TOF and the K_0 from three different methods of calibration compared versus traditional IMS data. In addition the stability of the TIMS device was studied by monitoring the elution potential of four peaks of known K_0 over a 10 hour period to determine the stability of the calibration.

Results:

TIMS analysis of the 622 (CAS NO.：186817-57-2), 922 (CAS NO.：58943-98-9), 1222 (CAS NO.：16059-16-8), and 1522 (CAS NO.：3830-74-8) m/z peaks from Agilent low concentration tune mix (G1969-85000) were used to investigate the stability of calibration over a ten hour period. A variance of less than 2% of the value of V_{tunnel} for a given peak was observed. Data from these calibrants were then used to test three approaches for conversion of elution potential to K_0. In the first approach a simple calibration is made from a single run of low concentration tune mix. A linear plot is then fitted of $1/V_{tunnel}$ vs. K_0. Unknown K_0 values can then be determined if pressure, temperature, and gas composition remain fixed. This simple approach also assumes external factors such as post TIMS transit times are identical for all ions. While extensive study of post TIMS transit times hasn't been completed, transit times on the order of 10% of the analysis time are typical and are subject to both the mass-to-charge ratio and K_0 of ions. To reduce the effect of post TIMS transit times a second approach in which a delay is added into the scan, and calibration carried out at five different delay times to allow extrapolation back to zero delay. While this second approach reduces the impact of post TIMS times, it unfortunately has the limitation of only being valid for analysis of species over the same scan time and same scan voltage range. A third calibration is therefore proposed and being tested in which five different scan rates are used for the calibrants and unknowns. By using this approach both the post TIMS times and the effect of variable scan speeds are taken into account and should provide a highly accurate calibration method for TIMS analysis.

PTh-078
13:30 – 14:40

Enhancement of Nanowire Formation by Mixing Multiple Modified Amyloid Peptides: Mechanism and Application

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Keywords:
amyloid fibrils, peptide self-assembly, ion-mobility spectrometry, oligomer structure, functional nanowires

Novel aspects:
Formation of extended oligomer structure is drastically enhanced in our mixed peptide system that gives extremely long resulting fibrils.

Abstract:
Protein misfolding and aggregation that result in the formation of amyloid fibrils are associated with many diseases, such as Alzheimer's disease and transmissible spongiform encephalophaties. Amyloid fibrils have rigid filamentous structures that are several nanometers wide and a few micrometers long and are also formed from a variety of peptides *in vitro*. Thus, amyloid-forming peptides have been focused on as potential materials to form functional nanowires used in nanotechnology.

We have previously developed a method to control fibrillation to effectively obtain functionalized nanowires using Structure-Controllable Amyloid Peptides (SCAPs) , wherein various three-amino-acid residue units are attached at the N-termini of amyloid peptide. We also found that mixing multiple SCAPs with different units of Lys_3 (K_3-TTR) and Glu_3 (E_3-TTR) residues drastically enhances the fibrillation and gives extremely long fibrils that are far over 40 μm long. Various functionalized nanowires have been successfully prepared through the effective formation of probed fibrils formed from mixed SCAPs. In the current study, we utilized the ion-mobility spectrometry-mass spectrometry (IMS-MS) method to characterize the mechanism of mixing SCAPs on the fibrillation enhancement.

K_3-TTR, E_3-TTR and their 1 : 1 mixture were electrosprayed from acidic aqueous solutions. Ions pass through a drift cell for collision with helium buffer gas and their mass-selected collision cross-section are directly measured on a home-built instrument. In the mass spectra of K_3-TTR and E_3-TTR there were some oligomer peaks up to trimer. On the other hand, the 1 : 1 mixture had multiple oligomeric species up to pentamer. The peak position of the oligomeric species of the mixture was centered between those of K_3-TTR and E_3-TTR in the high-resolution mass spectra. Therefore, the formation of heterooligomers is more favorable than those of the homooligomers. Interestingly, collision cross-section analysis revealed a dramatic structural difference between the homo- and hetero-oligomers. The oligomers of the 1 : 1 mixture dominantly form extended fibrillar structure even at the dimer level whilst species with this structure are absent or minor in K_3-TTR and E_3-TTR. These results show that mixing SCAPs controls the self-assembly ability of soluble peptides to form extended structure, and thus should also influence the formation of the extremely long fibrils.

In summary, it is suggested that mixing multiple SCAPs controls the structure of resulting fibrils. The length in particular should be controlled through altering self-assembly property of the peptide oligomers. Elucidating the relationship between the structures of oligomers and resulting fibrils will therefore give an important insight to tailor functionalized fibrillar assembly.

PTh-079
11:10 – 12:20

Internal energy of ions in travelling wave ion mobility spectrometry: dependence on instrument parameters and ion properties

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Mass Spectrometry Laboratory, University of Liege

Keywords:
Ion temperature, T-wave, Ion mobility

Novel aspects:
This work addresses the quantification of the internal energy of ions inside TWIG mobility cells on commercial instruments and show how this energy evolves with ion mass and physical properties.

Abstract:
Ion mobility spectrometry (IMS) separates ions according to their tridimensional shape in the gas phase. It relies on electric fields that drag ions inside a cell filled with gas ; the larger are the ions, the greater is the resistance and the longer is their travelling time. This technique can be coupled with mass spectrometry (MS) in order to achieve a two-dimensional separation. Currently available commercial IMS-MS instruments are based on the travelling wave technology. T-waves involve high electric fields, which induce fast ion velocities inside the cell. Based on these properties, Shvartsburg and Smith[1] arose potential ion heating due to inelastic collision with the gas. We experimentally proved this point by probing the fragmentation of *p*-methoxybenzyl pyridiniumions on the first-generation SYNAPT spectrometer (Waters, Manchester, UK) .[2] We present new data acquired on the SYNAPTG 2 HDMS, and heating value for systems of different mass, size and number of vibrational degrees of freedom.
Experiments were carried out both on a Synapt HDMS and Synapt G 2 HDMS (Waters, Manchester,UK) . The product ion spectra (ratio between parent and fragment) and ion mobility spectra of three different benzylpyridinium ions as well as small proteins were recorded as a function of the wave height, wave speed, gas pressure, and the nature of the gas (He, N_2, Ar, CO_2) . The arrival time distribution of the fragment was deconvoluted to distinguish fragments formed before, inside, or after the mobility cell. Matching calculations based on integrated RRKM or Arhénius equations with experiments, we obtained the vibrational internal effective temperature $T_{eff,vib}$ of the parent ion.
We studied the heating of the p-methoxybenzyl pyridiniumion in both the Synapt G 1 and G 2 for ideal conditions of separation. On the Synapt G1, we found a temperature $T_{eff,vib}$= 555 ± 2 K (wave height = 9 V, wave speed = 600 m/s, *p* (N_2) in IMS = 0.55 mbar) . On the Synapt G2, the parameters were different because of the mobility cell architecture (wave height = 35 V, wave speed = 1000 m/s, *p* (N_2) in IMS = 2.3 mbar) and we found $T_{eff,vib}$= 700 K. This value lies between those obtained for *p*-chlorobenzylpyridinium (720 K) and *p*-tertbutylbenzylpyridinium (630 K) . For leucine-enkephalin, we obtained $T_{eff,vib}$=420 K, which is compatible with the estimation of Merenbloom *et al.*[3] These results indicate a dependence of $T_{eff,vib}$on the ion size. The relevant physical parameters could be the ion collision cross section (CCS) and/or the number of vibrational degrees of freedom (DOF) .
On both instruments, we found a correlation between the ion speed v = $KE = K_0N_0E/N$ and the ion temperature : the amount of transferred energy increases with ion speed. This translates into an influence of the wave height (*WH*, influencing the field *E*) and gas pressure (*p*, influencing the gas number density *N*) on $T_{eff,vib}$. The influence of the wave speed (*s*) on $T_{eff,vib}$ behaves as if the effective electric field E_{eff} undergone by ions decreases when *v* becomes higher than *KE*. Thismeans that it becomes harder for ions to keep up with the field changes as they pass from one wave to the next one.
We found that $T_{eff,vib}$could be predicted from the apparent ion drift velocity $v_d = L/t_d$ (L is the length of the cell, t_d is the drift time) on the SYNAPT G 2 whereas a direct correlation between the drift speed *v* and $T_{eff,vib}$ could be established on the SYNAPT G1.

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PTh-080 An ion mobility mass spectrometer with an axial sector TOF analyzer

13:30 – 14:40

Alexey A Sysoev, Alexander A Sysoev, Sergey S Poteshin, Denis M Chernyshev

National Research Nuclear University MEPhI, Moscow, Russia

Keywords:
ion mobility spectrometry, time-of-flight mass spectrometry, atmosphere pressure ionization

Novel aspects:
Combining high resolving power IMS and orthogonal acceleration sector TOF mass analyzer can allow compactness and better ion sampling efficiency for orthogonal accelerator comparing existing reflectron based designs.

Abstract:
Recently developed ion mobility mass spectrometer is discussed. The instrument includes atmosphere pressure ionization ion sources, high resolution drift tube ion mobility spectrometer, differential pumping interface and axial sector TOF mass analyzer with helical path of ions. Data collection is performed using fast ADC based integrating transient recorder that allows real time data collection.
High resolving power IMS used here was previously described as a part of IMS/MS with triple quadrupole analyzer [1] and an instrument with Faraday plate detector [2] . Novelty of described approach is based on combining high resolving power IMS and orthogonal acceleration sector TOF mass analyzer. Main advantages of mentioned approach are compactness and better ion sampling efficiency for orthogonal accelerator comparing existing reflectron based designs.
The instrument was tested in positive ion mode using IMS standards (2,6-di-tert-butylpyridine, tetrapropylammonium iodide, tetrapentylammonium iodide, tetraoctylammonium bromide) and antibiotics (amoxicillin, ampicillin, lomefloxacin, ofloxacin) . Both methanol and acetonitrile were used as solvents.
Primary tests has shown that the combination of the IMS and an orthogonal acceleration axial sector time-of-flight mass analyzer can allow obtaining 100 mobility resolving power and 2000 mass resolving power. Mobility / mass distribution data collection time was varied between 3 and 100 seconds. For mentioned parameters nM and sub-nM range limits of detection were shown for studied compounds in electrosprayed liquid samples.

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PTh-081
11:10 – 12:20

Energy-resolved ion-mobility tandem mass spectrometry: a new tool for probing gas-phase isomerization and fragmentation of small molecules

Yayoi Hongo[1], Takemichi Nakamura[1], Takae Takeuchi[2]

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Keywords:
Energy-resolved ion-mobility tandem mass spectrometry (ER-IMS/MS 2) ; fragmentation ; isomerization ; small molecules ; structural characterization

Novel aspects:
Energy-resolved ion-mobility tandem mass spectrometry (ER-IMS/MS 2) was shown to be a useful tool for characterizing small molecules' ion isomerization and fragmentation in mass spectrometers.

Abstract:
We introduce energy-resolved ion-mobility tandem mass spectrometry (ER-IMS/MS^2) as a means to detect structural changes in small molecules that may occur in the gas-phase inside mass spectrometers. The ER-IMS/MS^2 approach enabled us to detect structural changes (or isomerization) of gas-phase ions prior to and/or in parallel to fragmentation that give *m/z* changes. A few examples have shown that compound specific isomerization is detectable by ER-IMS/MS^2; valuable information for structural characterization of small molecules can be obtained.

In the past decade, IMS of mass-resolved ions has become widely recognized and applied as a means to characterize gas-phase 3 D-structures of a wide range of large biomolecules. On the other hand, unlike many large biomolecules in the native states, ions derived from diverse small molecules (<2,000 Da) are not necessary to have well defined stable 3 D structures in the gas-phase. In case a certain level of conformational flexibility exists, simple correlation between peaks separated by IMS and isomeric ion structures may not be established.

Tandem mass spectrometry (MS^2) has been one of the most powerful and versatile means to probe structure of gas-phase ions through fragmentation chemistry. One obvious limitation of MS^2 is, however, its limited ability to detect isomerization of ions in the fragmentation process. Traveling-wave ion mobility spectrometry (TWIMS) offers us unique opportunities to looking at not only mobility of mass-resolved ions but also fragmentation process of the mass-resolved ions, simultaneously. We took advantage of this to develop ER-IMS/MS^2 strategy for characterization of small molecules. By linking structural (shape) change and fragmentation chemistry/energetics observed in ER-MS^2 experiments, both isomerization of precursor ions upon collisional activation and generation of isomeric product ions can be investigated in conjunction with computational chemistry.

A Synapt G 2 instrument (Waters, Manchester) was used for all the experiments. Small-molecule samples were introduced into the ESI source with infusion mode or LC/MS mode and precursor ions of interest were selected at Q1. Data was acquired with "IMS-On " mode (nitrogen in the IMS device) at various collision energy (CE) settings in the 1 st (pre-IMS) and/or 2 nd (post-IMS) collision cell, both filled with argon.

Among the various analytes tested including bioactive natural products and their derivatives, some showed clear signature of CE-dependent isomerization of precursor ions. Some showed CE-dependent generation of isomeric product ions, as well. For example, protonated molecule of erythromycin appeared to pops off one of the sugar moiety with no noticeable structural change in the aglycone. The following dehydration reaction appeared to give isomeric product ions. Both CE-dependent isomerization of precursor ions and isomer-dependent fragmentation processes were observed in case deprotonated molecule of folic acid was selected as the precursor.

As isomerization of gas-phase ions are structure specific chemistry in general, ER-IMS/MS^2 strategy is a promising tool for MS-based structural characterization of small organic molecules.

PTh-082
13:30 – 14:40

Separation of Peptide Sequence Isomers by Capillary Electrophoresis Coupled with Ion Mobility Mass Spectrometer

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Keywords:
Ion Mobility, Capillary Electrophoresis, Peptide Sequence Isomers, Separation

Novel aspects:
Peptide sequence isomers were separated by Capillary Electrophoresis coupled with Ion Mobility Mass Spectrometer

Abstract:
Sequence inversions are a major class of isomers of proteolytic peptides which are important for protein identification. Using mass spectrometry (MS) for separation of the equal mass isomers is limited. Capillary electrophoresis (CE) was proven as high-power technique for separation of very close molecules by their size or shape and could be effective for isomer separation from the mixtures. Ion mobility separation (IMS) device coupled to mass spectrometer is the powerful tool for detection and separation of isomeric peptides. But it is still a problem to separate isomers with the short reversed sections of the overall sequence.
In this study we compared two techniques for separation of three synthetic peptides with the short reversed AVPI sections : AVPIGGGAVPIG (Direct-Direct, DD) , AVPIGGGIPVAG (Direct-Reverse, DR) and IPVAGGGIPVAG (Reverse-Reverse, RR) . The alanine residues were methylated. In liquid phase the separation was made by CE with the ultraviolet (UV) optical detection. A mobility evaluation of ionized isomeric molecules in gas phase was made by IMS with tandem MS (MSMS) detection. The experiments with CE-IMS-MSMS instruments connected online were carried out to compare the separation capabilities of these techniques.
The CE-UV experiments showed separation of the peptides from mixtures up to 20 % of peak height above the baseline. The separation occurred in acidic CE buffers only and increased with the lowering of the pH value. The DD peptide migrated in capillary more slowly whereas the DR and RR peptides moved faster and were not separated from each other under all tested conditions.
The mobility of the single charged positive ions was measured by IMS-MSMS separately for all peptides. The difference between DD and DR was 1.6 %, between DD and RR was 5.9 %, between DR and RR was 4.2 %. The most different DD and RR peptides were separated from the mixture by IMS-MSMS at 70 % of peak height above the baseline.
Online combination of the methods showed the overlay of the CE migration time and the IMS mobility parameters measured in one experiment with MSMS detection of the separated components.
The difference in ion mobility of the isomers in gas phase as well as in solution depends on their charge state and charge distribution that affects on folding. In highly acidic solution the differences in the right part of the sequence cause changes in the folding. Whereas the left part of the sequence has greater effect on mobility of single protonated peptides in gas phase.

PTh-083

11:10 – 12:20

Challenges in high resolution and accuracy mass measurement for drug discovery and development using FT ICR and Orbitrap mass spectrometers

Zenzaburo Tozuka

Osaka University

Keywords:
FTICRMS, OrbitrapMS, AMS, MIST, Microdose

Novel aspects:
high sensitive, accurate structure elcidation of comprehensive drug metabolites using nanoLC-LTQ orbitrap, FT ICR MS and AMS to solve safety testing of drug metabolites in microdose clinical study

Abstract:
I challenged in high resolution and accuracy measurement of LTQ FT ICR MS during development in Bremen to solve the estimated two different fragment pathways of FK228 (Istodax) having same mass but different exact mass and different composition of atoms 1). On the base of their composition analysis by FT ICR MS, I could elucidate the structure of *in vitro* 14 metabolites and *in vivo* 8 metabolites of FK228 using SRM datadependent exclusion MSn measurements 2). Challenges to elucidate structure of 9 metabolites of Nicardipine for microdose clinical study (MDCS) 3) and determine the 12 biomarkers of breast cancer by shot gan analysis in combination with their antibody analysis using brest cancer tissues (International Patent) and in target proteome analysis of CYP 3 A 4 promoter factors binding their DNA segments (ISSX abstract), and CYP 3 A 4 - Raloxifene active metabolites adduct 4) were succeeded by two LTQ FT ICR MS in our laboratory. According to safety testing of drug metabolites (MIST) guidance, the quantitative and qualitative analysis of comprehensive metabolites of 14C-tolbutamide (TB) without standard samples was succeeded by UPLC-RI-LTQ Orbitrap MS 5). MIST items of 14C-TB and 14C-acetoaminophen 6) in MDCS were conducted by AMS and nanoLC-LTQ Orbitrap that was possible to elucidate the structure of metabolites using MDCS samples (urine and plasma) in addition to their quantitative analysis.

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PTh-085

11:10 – 12:20

Silicon speciation in petroleum products using a multi-technical approach by Mass Spectrometry for a better understanding of catalyst poisoning

Fabien Chainet[2], Jeremie Ponthus[1,2], Charles-Philippe Lienemann[2], Marion Courtiade[2], Olivier François X Donard[3]

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Keywords:
silicon speciation, catalyst poisoining

Novel aspects:
Due to the thermal degradation of PDMS in the presence of hydrocarbons, more than 100 silicon species were highlighted and could act as a poison on hydrotreatment catalysts

Abstract:
Silicon speciation is of considerable importance in the oil and gas industry due to the negative effect of its relative species on the performance of hydrotreatment catalysts. In petroleum products, silicon mainly comes from the use of antifoaming such as polydimethylsiloxanes (PDMS) to avoid emulsions in the different processes such as cokefaction, visbreaking, steamcracking or distillation and to enhance the crude oil recovery from the reservoir. PDMS, consisting in a structural unit of $(CH_3)_2$-Si-O-, has excellent properties of low surface tension and initial great thermal stability. Due to the high temperature applied in thermal cracking processes, PDMS degrades around 300 ° C and mainly generates cyclic siloxanes (D_n) with also many different silicon species in petroleum products that can affect the performance of catalysts.
In the oil and gas industry, the possible reactivity of hydrocarbon radicals with PDMS degradation products, was never reported and the chemical nature of these related silicon species must be determined. Up to now, these issues have been well identified but only addressed through the total silicon determination. These results have displayed total concentration levels ranging from $\mu g.kg^{-1}$ of Si up to some $mg.kg^{-1}$ of Si in petroleum products measured by elemental techniques. Moreover, the complexity of petroleum matrices, the wide variety of compounds concerned combined with possible contamination problems occurring during silicon analysis has hampered the development of speciation studies.
A very innovative and complete multi-technical approach based on MS techniques was developed and applied to identify and quantify silicon species. PDMS degradation samples, produced under thermalcracking of hydrocarbons on a pilot plant and several naphtha and gasolines coming from different refining processes were investigated. For low molecular weight silicon compounds, gas chromatography (GC) hyphenated to MS in single ion monitoring (SIM) was performed in gasolines. Cyclic siloxanes were confirmed as the major degradation products with trace of linear siloxanes. However, suspected unknown silicon compounds cannot be detected as their structures and fragmentations patterns remained unknown. To overcome this challenge, FT-ICR/MS was performed in positive electrospray mode (ESI) and allowed the characterization by their raw formula of more than 50 new silicon compounds with several unsaturations in naphtha and gasoline samples. To obtain a quantification and a structural identification of these compounds, GC-ICP/MS providing a specific detection combined to GC-GC/MS addressing the chemical structure were carried out both in PDMS degradation samples and in naphtha and gasolines. Finally, MS/MS experiments were also carried out to confirm the suspected chemical structures.
More than one hundred silicon species were highlighted in this work. Silicon compounds present in PDMS degradation samples and in gasoline were compared and confirmed the correct representativity of the evaluated conditions of PDMS degradation samples. The presence of PDMS, intermediate polymers and low molecular weight silicon compounds could explain the distribution of silicon amount in the different cuts of petroleum products and probably the poisoning effect on hydrotreatment catalysts.

PTh-086

13:30 – 14:40

Development and validation of high resolution liquid chromatography-time of flight method of Bacopaside-I and metabolites for pharmacokinetic study

Sontaya Sookying[2], Kornkanok Ingkaninan*[2], Dumrongsak Pekthong[3], Sarawut Oo-puthinan[3], JIE XING[1], Zhaoqi Zhan[1]

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Keywords:
Bacopaside-I, quantification, validation, metabolite, pharmacokinetics

Novel aspects:
Quantitative analysis of Bacopaside I by high resolution LC-MS (TOF) method and metabolite identification.

Abstract:
*Corresponding author

Introduction

Bacopa monnieri (L.) Wettst. (Brahmi) is an Ayurvedic medicine plant used for centuries as a memory enhancer. There have been several studies on biological effects of this plant for a therapeutic potential in treatment and prevention of neurological diseases and improvement of cognitive processes. Bacopaside I ($C_{46}H_{74}O_{20}S$) , a dammarane-type triterpenoid saponin, is one of the main active components of the plant and, therefore, it has been produced and used in biological and therapeutic studies. Various analytical method such as HPTLC, ELISA and HPLC methods were used to determine bacopasides I and other active components in the plant extracts as well as biological samples. However, so far LC/MS method for quantitative analysis of Bacopaside I in biological samples has not been reported. We report for the first time the development and validation of a high sensitivity LC-MS (TOF) method, aiming for pharmacokinetic study of bacopasides I and identification of its metabolites produced *in vitro* and *in vivo*.

Experimental

Rat urine and feces were used as the biological matrix for method development and validation of bacopaside I quantitative analysis. Liquid-liquid extraction method was employed in sample extraction and purification. The LC-MS method used is based on high resolution TOF which is suitable for both quantitative analysis of Bacopaside I in biological samples and qualitative analysis to identify any metabolites formed. An LCMS-IT-TOF (Shimadzu Corporation, Japan) has been used in this study

Results and Discussion

This study was carried out in three stages, development and optimization of a high resolution LC/MS (TOF) method, validation trials and identification of metabolites of bacopaside I from various *in vitro* hydrolysis samples using a MetID Solution program. A gradient elution method using a Kinetex C18 column (1.7 um, 50 mmL x 2.1mmID) was developed and optimized. It was found that both target compounds (Bacopaside I and an internal standard, Digitoxin) showed very strong mass peaks (m/z 977.4387 and 763.4243) in negative ESI mode. A high resolution MS and MS/MS method were established to obtain best sensitivity and highest reliability of analysis.

The performance of the method has been evaluated systemically using extract samples prepared from rat urine and feces. The linearity (r 2) was 0.9985 for a range of from 4.8 ng/mL to 380 ng/mL. The specificity and matrix effect were studied and it was found that the method specificity was highly reliable due to the use of accurate mass (target m/z +/- 50 ppm) on the high resolution TOF mass spectrometer. Matrix effect was not obvious under the LC/MS condition. The LOQ of the method was lower than 4.8 ng/mL. The reproducibility at this concentration level (spiked into urine) was 8.7 % (RSD of concentration, n= 6) . The recoveries of bacopaside I spiked into urine were 91.1 % and 107.1 % for three concentration levels (50, 80 and 100 ng/mL) . However, the recovery of feces extract samples was found lower than that of urine samples at 40~70%. Further study to improve the recovery of bacopaside I in feces samples is undergoing. The method has been applied to *in vitro* hydrolysis samples. Some metabolites were found from the acid hydrolysis samples by using MetID solution program, which is based on an editable biotransformation database.

Conclusions

A high resolution LC-TOF method for quantitative analysis of bacopaside I in biological samples has been established on LCMS-IT-TOF. The method can be used for pharmacokinetics study and it is also capable of identification of metabolites of bacopaside I by using a MetID soution

Poster Session

Thursday, 20th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 37: Challenges in High Resolution and High Accuracy Mass Measurement Mass Spectrometry

PTh-087

11:10 – 12:20

Characterization of Humic and Fulvic acid fractions separated by size exclusion chromatography and application of 15T FT-ICR MS

Min-Hui Son, Yoon-Seok Chang

POSTECH, Pohang, Korea

Keywords:

Humic and Fulvic acids, Size Exclusion Chromatography (SEC) , FT-ICR MS

Novel aspects:

Humic and fulvic acid fractions separated by SEC was analyzed with 15T FT-ICR MS and this is the first study that humic and fulvic acid fractions were examined in Korea.

Abstract:

Understanding the characterization of molecular level of humic and fulvic acid, which has enormous complexity and high molecular weights, is still very difficult in spite of the application of high resolution mass spectrometry or various ionization techniques. For this reason, separation techniques, such as size exclusion chromatography (SEC) or capillary electrophoresis (CE), and ultra high resolution mass spectrometry (FT-ICR MS) have been attempted to the characterization of humic and fulvic compounds in many studies. In spite of their important physicochemical properties, however, they were not completely characterized at molecular level due to the tremendous complexity of their structures. In this study, humic substances including humic and fulvic acid were separated by size exclusion chromatography (SEC) and analyzed with negative ion mode electrospray ionization (ESI) ultrahigh resolution mass spectrometry (15T FT-ICR MS) . Suwannee river humic acid (SRHA) and fulvic acid (SRFA) standards obtained from IHSS (International Humic Substance Society) were used for molecular weight distributions. In order to better interpretation of mass spectra, humic and fulvic acid unfractionated were also analyzed. Size exclusion chromatography has been widely used as separation technique, but 15T FT-CR MS is not common as conventional high mass spectrometry.

PTh-088

13:30 – 14:40

Dependence of Field Evaporation in Three-Dimensional Atom Probe on the Crystallographic Orientation

Mahito Shimizu, Masato Morita, Yuya Hanaoka, Masanori Owari

The University of Tokyo, Tokyo, Japan

Keywords:

three-dimensional atom probe, field evaporation, crystallographic orientation, time-of-flight, high mass resolution

Novel aspects:

We newly discussed field evaporation dependence on the crystallographic orientation of the sample surface for improving three-dimensional atom probe tomography.

Abstract:

Understanding of relationship between material property and microstructure is one of the main topics of materials science. For achievement of this challenge, knowledge of materials structure is required at the sub-nm scale. Historically, spatial resolution of available microstructural analysis techniques was insufficient for elucidation of the role of materials elements to materials properties. More recently, however, the spatial resolution limits have been amazingly improved by the advent of microstructural analysis techniques such as three-dimensional atom probe (3DAP).

3DAP is a field ionization microscope equipped with a time-of-flight mass spectrometer (TOF-MS) and a position-sensitive detector (PSD). The samples used in 3DAP are usually shaped into sharp needle geometry whose tip radii are approximately 100 nm. In 3DAP, when strong electric field is applied to the tip of a sharp needle sample by high standing voltage (DC) and a voltage pulse or a laser pulse, the atom which has the smallest number of nearest neighbor atoms ionizes in sequence. Then, the positive ion is removed from the surface of specimen and travels to a PSD accelerated by the electric field. This ionization is called field evaporation. The elemental identity of the ion is determined with a TOF-MS. The lateral position of each atom in the sample is determined from the position arrived on a PSD. The depth coordinate from the tip of the sharp needle sample is determined from the position in the field evaporation sequence. A sub-nm scale three-dimensional image of the specimen is reconstructed from these data in the virtual space by the computer algorithm.

In order to improve analysis accuracy of 3DAP tomography, knowledge about influential factor affecting field evaporation is needed. In this study, we noted the effect of crystal structure on field evaporation. As samples, Tungsten (W), which had body-centered cubic lattice, was used. W needles with different crystallographic orientations were analyzed by 3DAP. In the case of the sample with [110] orientation, field evaporated ions were mostly W^{3+} and W^{4+}. On the other hand, in the case of the [100] sample, field evaporated ions were mostly W^{3+}, W^{4+} and W^{5+}. It was revealed that field evaporated ions are dependent on the crystallographic orientation of the sample surface. In addition, it was found that the accuracy of 3DAP tomography was also dependent on the crystallographic orientation of the sample surface. In the atomic scale three-dimensional image of the [110] sample, crystal planes could be observed regardless of depth. On the other hand, in the atomic scale three-dimensional image of the [100] sample, crystal planes could be observed only at the surface.

These two facts, both field evaporated ions and 3DAP tomography accuracy were dependent on the crystallographic orientation, were very interesting. By considering the relationship between these two facts, we will discuss how the crystallographic orientation of sample surface affects the field evaporation mechanism in 3DAP.

PTh-089
11:10 – 12:20

Theoretical Verification of a Strategy for Determining Elemental Composition Based on Ultrahigh-resolution Mass Spectrometric Data

Tatsuhiko Nagao[1], Daichi Yukihira[1], Yoshinori Fujimura[1], Kazunori Saito[2], Daisuke Miura[1], Hiroyuki Wariishi[1]

[1]Kyushu University, Fukuoka, Japan, [2]Bruker Daltonics K.K., Yokohama, Japan

Keywords:
elemental composition, isotopic fine structure, ultrahigh-resolution, FT-ICR-MS, heuristic rule

Novel aspects:
This study highlighted potential superiority of using ultrahigh-resolution mass spectrometry and additional constraint rules for identifying unique correct elemental composition independent of standard compounds.

Abstract:

Background

Identifying metabolites with mass spectrometric data requires comparisons with spectral databases or reference standard spectra, despite the fact that a large proportion of biological metabolites are not commercially available. Thus, a metabolite identification method independent of standard compounds is indispensable technique for metabolomics.

Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS) has high mass accuracy and ultrahigh mass resolving power. Previously, we demonstrated the possibility of determining the elemental composition of metabolites using the peak area ratio of ^{13}C, ^{15}N, ^{18}O, and ^{34}S isotopic fine structure observed by FT-ICR-MS independent of standard compounds. In this study, we represented an algorithm to calculate elemental composition from mass spectrometric data automatically. Then, we validated the algorithm through simulations using *in silico*-generated 20,258 mass spectra to understand the effect of error size of isotopic peaks on elemental compositions obtained via the algorithm.

Results

To prepare *in silico* mass spectra of metabolites, molecular formula of metabolites was needed. First, 20,258 kinds of different elemental compositions comprising only C, H, O, N, P and S elements were selected from the original dataset including molecular weight and molecular formula of metabolites downloaded from http：//www.metabolome.jp/. To replicate mass spectrum of FT-ICR-MS *in silico*, theoretical mass and theoretical area ratio of isotopic peaks to monoisotopic peak were re-calculated for each entry. Theoretical monoisotopic masses were treated with 0.1 - 1 ppm random error to replicate the measured mass accuracy of FT-ICR-MS. Theoretical relative isotopic area was replicated by an opposite procedure of estimating the number of element. Then, random errors were added to relative isotopic areas with varying maximum errors from +10% to -25%. To compensate the random errors in isotopic area, estimated numbers for each element are enlarged by ±25 % safety factor, thereby generating ranged numbers of element. In the simulation of determining elemental composition, deprotonated peak ($[M-H]^-$) was taken into consideration for the negative ion mode, and proton- and alkali metal-adducted peaks ($[M+H]^+$, $[M+Na]^+$, $[M+K]^+$) for the positive ion mode. Simulations were performed for 100 times to average number of estimated elemental compositions.

Since isotopic area is fluctuant because of the space charge, the relationship between isotopic area error and the number of estimated elemental composition was investigated. Both in the simulation of negative and positive ion modes, the percentage of metabolites identified as a unique correct elemental composition was decreased from 61 % to 45 % and from 58 % to 38 % with diminishing maximum area error from +10% to -25%, respectively. Thus, approximately 50% of 20,258 metabolites were generated more than 2 candidates. These results indicated that using ^{13}C, ^{15}N, ^{18}O and ^{34}S isotopic fine structure is insufficient for limiting elemental composition as few as possible. To expand the percentage of metabolites identified as unique correct elemental composition, we added another constraint rules to the algorithm. First, since we found that either C_4 (48) or H_3NP (48.00031) was replaced with a complement one between a correct elemental composition and incorrect ones, we used oxygen/phosphor ratio (O/P ratio) to remove such incorrect candidates. The O/P ratio in KEGG compounds was investigated thoroughly ; thereby the O/P ratio was elucidated as greater than 2. By applying the O/P ratio, the percentage of metabolites led unique correct elemental compositions was increased to 60 % . Moreover, the valence check rule introduced by T. Kind and O. Fiehn (2007) was also effectively increased the percentage of metabolites obtained unique correct candidate up to 90%.

PTh-090
13:30 – 14:40

Analysis of Wedelia trilobata flower extract using Liquid Chromatography-Orbitrap Mass Spectrometry

Jhih-Yang Huang[1], Ean-Tun Alex Liaw[1], Chien-Kuo Han[2], Ming-Feng Chen[3], Jean Chih-Chun Huang[1]

[1]National Pingtung University of Science & Technology, Pingtung, Taiwan, [2]Asia University, Taichung, Taiwan, [3]National Cheng Kung University, Tainan, Taiwan

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Keywords:
Wedelia trilobata ; LC-Obitrap-MS ; antioxidant activity ; phenolics ; flavonoids

Novel aspects:
By using the advanced instrument such as Orbitrap mass spectrometer for accurate mass and high-resolution measurement, one can expect for simplified procedures, reduced analysis times and increased confidence in results.

Abstract:
Wedelia trilobata, categorized in the Heliantheae tribe of the Asteraceae family, is a plant widespread in subtropical countries such as Brazil, India, Burma, Ceylon, China and Japan. This kind of herbal plants has been reported in traditional Chinese medicine to show anti-inflammatory properties and used as a detoxifying agent for liver protection from toxicity. To fully understand the active compounds and their functionality mechanisms, systematic analyses are in need to provide more scientific evidence. In this study, we attempted to analyze the components in the ethanolic extract of the *Wedelia trilobata* flowers using liquid chromatography coupled with electrospray ionization/Orbitrap mass spectrometry (LC-Orbitrap-MS) , which is recently developed for accurate mass and high-resolution measurement. By using this advanced instrument, simplified procedures can be expected, as well as reduced analysis times and increased confidence in results for challenging analysis such as low level components in complex mixtures. In the meantime, we also assessed the antioxidant activity in terms of DPPH (2,2-diphenyl- 1 -picrylhydrazyl) scavenging capacity and the total phenolic and flavonoid contents of the extract using spectrophotometric methods. Our MS results suggest that the ethanolic extract may contain some phenolic compounds such as luteolin and quercetin and the phenolic gulcosidic conjugates. On the other hand, the results of antioxidant activity show that the extract at a concentration of 20 μg/mL could scavenge 50% DPPH radicals, while that for the concentration of the luteolin standard solution was 6.0 μg/mL. One milligram of the extract was found to have total phenolic compounds of 96.2 μg gallic acid equivalents and total flavonoids of 24.5 μg quercetin equivalents.

PTh-091 Relevance of Gas and Pressure for Thermalization of Cluster Ions in the Hexapole Ion Trap of a FT-ICR Mass Spectrometer

11:10 – 12:20

Jürgen H Gross, Doris Lang, Iris Mitsch

Heidelberg University, Heidelberg, Germany

Keywords:

cluster ions, mass calibration, ESI, MALDI, FT-ICR-MS

Novel aspects:

Proper selection of the buffer gas pressure is relevant for the mass range covered by cluster ion peaks of intensities useful for mass calibration on certain hybrid FT-ICR instruments.

Abstract:

Introduction

Cluster ions are quite useful to establish mass calibration of instruments equipped with desorption/ionization sources over a wide *m/z* range. In our laboratory, we are routinely using the abundant $[arginine_n+H]^+$ and $[arginine_nH]^-$ cluster ions [1,2] for external mass calibration in positive-ion and negative-ion ESI mode, respectively. Likewise, $[Cs_nI_{n-1}]^+$ and $[Cs_{n-1}I_n]^-$ cluster ions can be used for calibration purposes in MALDI operation. The caesium salt cluster ions are generated from caesium triiodide, CsI_3, in 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene] malonitrile (DCTB) matrix [3].

In hybrid quadrupole Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers, however, multiple ion-guiding and ion-trapping events occur prior to mass analysis. During a recent implementation of a liquid injection field desorption/ionization (LIFDI) ion source on this instrument, the enormous relevance of the type and pressure of the buffer gas collisions in the instrument's accumulation RF-hexapole ion trap were recognized, because the selection of these settings was decisive for the detection of the easy-to-fragment molecular ions of hydrocarbon species [4]. Thus, we studied the effect of argon versus helium buffer gas and their pressure on cluster ion distributions intended for mass calibration in ESI and MALDI modes.

Experimental

The experiments were performed on a Bruker Apex-Qe FT-ICR mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 9.4 T superconducting magnet. The analyzer of this instrument is a quadrupole-ICR hybrid comprising a RF-only hexapole (h1), a selection RF/DC quadrupole (Q), a second RF-only hexapole (h2), and a high voltage ion transfer flight tube to bring ions from h2 through the magnetic field gradient into the ICR cell. Typically, h2 is used for ion accumulation prior to ICR analysis. In tandem MS mode, h2 is also employed for collision-induced dissociation (CID) and subsequent accumulation of the resulting fragment ions.

Ions were accumulated in the collision hexapole for 0.1 to 0.2 s and then transferred into the ICR cell. The standard buffer gas argon and the lighter alternative helium were admitted to h2 at various pressures. Ions were excited and detected using standard settings as established in ESI and MALDI mode. Data acquisition was performed in broadband mode with 512k to 1 *M* data points. Typically, 32 to 64 transients were accumulated for one magnitude mode spectrum. External mass calibration was performed on the cluster ions. The instrument was controlled by the Bruker ApexControl software (V 3.0.0) and data analysis was performed using the Bruker DataAnalysis software (V.4.0).

Results and Discussion

Argon buffer gas served better for cluster ion thermalization in h2 than helium, a trend that is opposed to expectations derived from previous LIFDI experiments. The pressure of buffer gas is more relevant for the cluster ion distribution, in particular for $[Cs_nI_{n1}]^+$ and $[Cs_{n1}I_n]$ cluster ions. Abundances of cluster ions of intermediate *m/z* of about 1500 to 3000 were increased upon raising the buffer gas flow to the maximum (2.0 l/s) while high-mass clusters beyond *m/z* 5000 benefited from medium gas flow (1.0 l/s) into h2. Thus, proper selection of the buffer gas pressure is relevant for the mass range covered by cluster ion peaks of intensities that are useful for calibration.

References

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PTh-092

13:30 – 14:40

A quantitative analytical method for determining the levels of glucose-dependent insulinotropic polypeptides GIP1-42 and GIP3-42 in human plasma using LC-MS/MS/MS

Atsushi Miyachi[1], Nana Morimoto[1], Yuichiro Yamada[2], Ken-ichi Harada[3]

[1]SANWA KAGAKU KENKYUSHO CO.,Mie,Japan, [2]Akita University School of Medicine, Akita, Japan, [3]Meijo University, Nagoya, Japan

Keywords:
GIP, Asp-N, LC-MS/MS/MS, diabetes, plasma

Novel aspects:
Proteolysis of GIP by Asp-N endopeptidase, not trypsin, is useful for a stable simultaneous quantification of GIP 1-42 and GIP 3-42 in human plasma using LC-MS/MS/MS.

Abstract:
[Purpose]
Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino acid peptide hormone released from duodenal K-cells after the absorption of glucose or fat. Similar to its structurally related peptide glucagon-like peptide-1 (GLP-1), which is released from intestinal L-cells, GIP is considered to be an incretin hormone. In addition to its incretin effect, GIP is considered to increase the activity of lipoprotein lipase (LPL), which is a key enzyme of lipid metabolism, as well as to promote the glucose absorption of adipose cells in the presence of insulin; it is also associated with bone metabolism. However, some in vivo roles of GIP are still unknown. Therefore, the quantitative evaluation of GIP secretion and active GIP levels is important. Active GIP_{1-42} as well as GLP-1 is degraded rapidly by dipeptidyl peptidase-4 (DPP-4) to yield GIP_{3-42}, which is inactive. Therefore, the concentration of active GIP_{1-42} in peripheral plasma is very low.
Current methodologies used for peptide and protein quantification include immunoassays and mass spectrometric techniques. Immunoassays, though sensitive, lack the necessary selectivity for recognizing the distinction between peptides, their recombinant forms, metabolites, and posttranslational products. In many cases, suitable antibodies for each protein analyte may not be available. Suitable antibodies for active GIP_{1-42} are more unlikely to be obtained. Additionally, it is noted that the plasma GLP-1 levels measured using different immunoassay kits are not consistent. The pretreatment of plasma samples is reported to be important. To this end, analysis by liquid chromatography-mass spectrometry (LC-MS) is useful. Although there have been several reports about the quantification of GIP using LC-MS (/MS), there is no report on the sensitive quantification of GIP_{1-42} and GIP_{3-42} in a clinical trial using only small volume of plasma. In this study, we used an LC-MS/MS/MS approach for the simultaneous absolute quantification of GIP_{1-42} and GIP_{3-42} in human plasma.
[Method]
Synthetic standard GIP peptides were spiked into charcoal stripped human plasma. Stable isotope (15N/13C) -labeled GIP_{1-42} and GIP_{3-42} were used as internal standards. Peptide fragments of GIP_{1-8} and GIP_{3-8} obtained by digestion using Asp-N endopeptidase were selected as analytes. Since both the fragmented peptides did not contain methionine, which is oxidized easily, the sample stability during and after extraction was expected to be good. Protein precipitation (PP) was performed during sample pretreatment before the digestion, and solid phase extraction (SPE) was performed after the digestion. Samples were injected onto a capillary column (C18; 150 mm x 75 µm I.D.), and GIP_{1-8}, GIP_{3-8}, and internal standard peptides were detected by hybrid triple quadrupole/linear ion trap mass spectrometry (QqQ/LIT-MS) operated in positive electrospray ionization mode.
[Results and Discussion]
The lower limit of quantification (LLOQ) of the reported method was 1 pM for GIP_{1-42} and 10 pM for GIP_{3-42} using 200 µL of human plasma, which were similar to the LLOQ of the GIP obtained by an immunoassay. The accuracy and precision values indicated good reliability of the analytical method. We applied this method to a clinical trial for diabetic subjects treated with DPP-4 and alpha-glucosidase inhibitors. Our results suggested that the described methodology successfully determined the efficacy of DPP-4 inhibitor alone, alpha-glucosidase inhibitor alone, and in combination, based on the plasma levels of GIP_{1-42} and GIP_{3-42}.

PTh-093 Metabolomic Analysis of Autism Spectrum Disorders Using Saliva

11:10 – 12:20

KEIJI GAMOH, KAZUTAKA SUGAI

Kochi University, Kochi, Japan

Keywords:
Autism, Metabolomics, Saliva, LC/MS

Novel aspects:
Demonstration of the metabolomic analysis of saliva and the comparison of the metabolites between autism spectrum disorders and controls based on the liquid chromatography/mass spectrometry (LC/MS)

Abstract:
Objectives:
Elucidation of autism spectrum disorders based on the metabolomic analysis of biological substrates in saliva.
Development of liquid chromatography/mass spectrometric analytical methods for the metabolites in saliva using a reversed-phase separation mode and an electrospray ionization mode.
Investigation of comparison of the concentrations of metabolites between the autism spectrum disorders and controls.

Autism spectrum disorders (ASD) are a behaviorally defined group of neurodevelopment disorders characterized by impairments in social interaction and communication, and repetitive, overly focused behaviors. While the syndrome has been shown to be highly heritable, various theories have been presented suggesting both genetic and environmental factors, such as dietary or chemical exposures. Analytical chemical investigation of autism spectrum disorders based on liquid chromatography/mass spectrometric method has been proceeded. Our interests were targeted to the concentration of biological metabolites in saliva of autism spectrum disorders. In the present study we demonstrated the metabolomic analysis of saliva and the investigation of comparison of the metabolites between autism spectrum disorders and controls using a reversed-phase separation mode and an electrospray ionization mode of the liquid chromatography/mass spectrometry (LC/MS).

PTh-094

13:30 – 14:40

Affintiy - Mass Spectrometry: A Proteome Signature for Intra-Uterine Growth Restriction Reveals Pathological Protein Glycosylation Abberations in Umbilical Cord Blood

Manja Wölter[1], Claudia Röwer[1], Cornelia Koy[1], Ulrich Pecks[2], Michael O Glocker[1]

[1]Proteome Center Rostock, [2]Womens Clinic, University of Aachen

Keywords:

Affinity-MS, cord blood serum profiling, proteome signature, apolipoprotein C-III, protein glycosylation

Novel aspects:

Multiparametric MS data analysis of a proteome signature for IUGR describes deglycosylated Apo C-III derivatives as key markers for the first time

Abstract:

Intrauterine Growth Restriction (IUGR) affects about 3 to 8 % of all pregnancies. It is defined as a condition in that the fetus does not reach its genetically given growth potential, resulting in low birth weight. IUGR is an important cause of perinatal morbidity and mortality, thus contributing substantially to medically indicated preterm birth. The pathogenesis of IUGR still remains unclear. Analysis of complex gestation-related patho-physiological conditions like IUGR requires methods that can cope with the complexity of the samples. We here describe mass spectrometric profiling of umbilical cord blood serum proteins to identify disease-related protein alterations with impact in diagnostics and even prognostics of gestation-associated diseases.

Cord blood samples from 30 newborns (15 from normal pregnancies and 15 from IUGR cases) were taken post-natally from the umbilical cord vein. Serum was prepared using standardized protocols. Samples were processed using affinity-beads. After elution, protein solutions were spotted directly onto stainless steel MALDI targets and mixed with ferulic acid, each in duplicate. Protein mixtures were analyzed with a Reflex III MALDI ToF mass spectrometer equipped with the SCOUT source and delayed extraction in linear positive ion mode, using an acceleration voltage of 20 kV. Spectra were recorded in a mass range from 4-25 kDa, accumulating 900 shots per spectrum. Spectra evaluation was performed with the ClinProTools 2.2 software and in-house biostatistic analysis tools.

Protein mixtures of cord blood samples were subjected to affinity fractionation in order to remove highly abundant proteins. After affintiy enrichment, MALDI mass spectra showed on average more than 60 protein ion signals between m/z 4000 and 25,000. The six best differentiating ion signals were at m/z 8205, 8766, 13,945, 15,129, 15,308, and 16,001, forming a proteome signature with which we were able to distinguish between IUGR and inconspicuous controls. Of note, apolipoprotein C-III_0 (m/z 8766, lacking glycosylation) was found more abundant in the IUGR samples, irrespective of gestational age, whereas other apo C-III forms with different glycosylation structures were not. Apo C-III is involved in triglyceride metabolism, which itself is discussed to be of importance in IUGR pathogenesis.

To assign ion signals to specific proteins, samples were subjected to SDS PAGE followed by PMF. We identified apolipoprotein C-III together with other apolipoproteins (apo A-I, A-IV) , fetuin A, and others. Identification of apo C-III was confirmed by MS/MS analysis. The MS/MS spectrum of the precursor ion at m/z 1196.61 showed a characteristic fragmention signal at m/z 638.37 from a D-G cleavage, together with other specific fragment ion signals, confirming the presence of the amino acid sequence 41-51from apolipoprotein C-III.

Furthermore, apo C-III concentrations in serum were measured by ELISA and show that protein abundance differences between IUGR and control cord blood samples can be determined reliably using an affinity-enrichment procedure combined with MALDIMS profiling. Mass spectrometry-based multiparametric analysis of cord blood samples is capable of differentiating individual samples from the IUGR group from those of the control group with high confidence.

In conclusion, we suggest (i) apolipoprotein C-III_0 as a key-marker of the IUGR proteome signature, and, that (ii) subtle alterations in glycosylation need to be considered for understanding the pathomechanisms in IUGR.

PTh-095
11:10 – 12:20

Automated Analysis of 25-hydroxyvitamin D2 and D3 with APCI-MS/MS Coupled with ZIVAK ONH-200BD Sample Preparation System

MURAT CELIK, HASAN OZGEN

ZIVAK TECHNOLOGIES, KOCAELI, TURKEY

Keywords:
25-hydroxyvitamin-D3, 25-hydroxyvitamin-D2, automated sample preparation, tandem mass, Zivak

Novel aspects:
The first automated MS analysis of 25-hydroxyvitamin D 2 and D 3 was made with ZIVAK ONH-200BD automated sample preparation system without any human control for reducing user errors.

Abstract:
Introduction

The measurement of serum concentrations of 25-hydroxy vitamin D 2 and D 3 is the most accurate way to determine vitamin D status in human body. 25-hydroxy metabolites of vitamin D, plays a critical role in controlling calcium and phosphate levels in the body. Quantitation of these metabolites is very important because of their clinical significance in variety of disorders like many chronic conditions and latency diseases, osteomalacia and rickets disease in children.

Because of their importance, analysis methods of 25-hydroxy metabolites must be reliable and sensitive. The conventional methods based on HPLC-UV has disadvantages for sensitivity, specificity and analysis time. On the other hand, the methods based on immunoassay has some specifity problems. As a result of these conditions, interest in LC-MS/MS methods growing during recent years.

Methods

A reliable, rapid and sensitive LC-MS/MS method with automated sample preparation system for analysis of 25-hydroxy metabolites was developed. Standarts, calibrators, controls and reagents of ZIVAK 25-hydroxyvitamin D 2 -D 3 LC-MS/MS Analysis Kit kindly provided by ZIVAK Technologies, Turkey. All sample preparation steps below was made by ZIVAK ONH-200BD automated sample preparation system without any human control for reducing user errors in sample preparation.

500 ul of serum samples pipetted into sample preparation vials. 400 ul of Reagent 1 including salt solution in appropriate concentration added and mixed serially by vortex unit of sample preparation system. Then, 400 uL of deuterated 25-hydroxyvitamin D 3 internal standard mixture in organic solvent added mixed serially by vortex unit of sample preparation system. After centrifugation at 5.000 rpm for 5 min, 50 ul of upper phase injected to LC-MS/MS system. Deionized water and methanol with 0,1% formic acid were used as mobile phases for gradient elution. 50x4.6 mm reversed-phase HPLC column used for chromatographic separation. Analysis run time was 6 minutes. Confirmation and quantification ions for all analytes and internal standards determined in APCI (+) scan mode. ZIVAK TANDEM GOLD LC-MS/MS system used for analysis.

Preliminary Data

Recovery for analytes were between 97%-105%. Linearity from 2,5 to 400 ug/L for both analyte. Correlation coefficients were 0,9989 and 0,9995 for 25-hydroxyvitamnin D 2 and D 3 respectively. Limit of detection for 25-hydroxyvitamin D 2 was determined as 1 ug/L while limit of detections for 25-hydroxyvitamin D 3 was 0.8 ug/L. Coefficients of variability values for 25-hydroxyvitamin D 2 and D 3 were 2,4 and 2,7% for intra-assay and 3,4-3,6% for inter-assay. No interferences was found for these analytes.

The NIST SRM 2972 sample was measured with this automated system and the results were found in median range for both analyte.

PTh-096

13:30 – 14:40

A LC/MS/MS Method Enables Simultaneous Detection of Diagnostic Biomarkers of Alkaptonuria, Ornithine Carbamoyltransferase Deficiency and Neuroblastoma Disease

Chien-Chen Lai[1], Wei-Yi Hsu[2], Fuu-Jen Tsai[2]

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Keywords:
LC/MS/MS ; Alkaptonuria ; Ornithine Carbamoyltransferase Deficiency ; Neuroblastoma ; Creatinine ; Screening

Novel aspects:
Creatinine and the metabolites in urine from patients with alkaptonuria, OCTD, or neuroblastoma were quantified in one-run using LC/MS/MS. The level of creatinine was to normalize the urine sample differences.

Abstract:
In this study, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) method was developed for simultaneous quantification of diagnostic markers of neuroblastoma, ornithine carbamoyltransferase deficiency, and alkaptonuria in urine, namely homovanillic acid (HVA) /vanillylmandelic acid (VMA) , orotic acid (OA) , and homogentisic acid (HGA) . After sample preparation, which involved only the dilution procedure, samples were quantified by LC/MS/MS. Full-scan MS/MS mode enabled the urinary markers to be quantified with a high degree of specificity and sensitivity. Rather than using a separate enzymatic method to normalize the concentration of creatinine in urine, we quantified the level of creatinine in urine in one LC/MS run. The limits of detection were 10 μg L^{-1} for HGA, 25 μg L^{-1} for HVA/VMA, and 50 μg L^{-1} for OA with a single-to-noise ratio of 3 ; the limits of quantification were 50 μg L^{-1} for HVA and HGA, 100 μg L^{-1} for VMA, and 250 μg L^{-1} for OA. Multiple calibration curves exhibited consistent linearity and reproducibility. The linear dynamic range of quantification of the analytes covered 2 to 3 orders of magnitude, depending on the analyte. The relative standard deviation of the developed LC/MS/MS method was less than 4 % for the intra-day validation and 10% for the inter-day validation. The results show that our LC/MS/MS technique is a highly sensitive and rapid method for screening for urine biomarkers that are diagnostic of three rare metabolic diseases.

PTh-097
11:10 – 12:20

Development of a high sensitive quantitation method for serum C-peptide by isotope-dilution mass spectrometry

Tomoya Kinumi, Mari Goto, Akiko Takatsu

National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan

Keywords:
Isotope-dilution mass spectrometry, C-peptide, Immunoaffinity purification

Novel aspects:
We first established the precise measuremt of low concentration of serum C-peptide by mass spectrometry using combination of chemical modification and immunoaffinity purification.

Abstract:

Introduction

Human C-peptide is a 31-mer peptide which is secreted into the blood from beta-cell in the pancreas where pro-insulin is cleaved into insulin and C-peptide during post-translational processing. Human C-peptide concentrations in blood plasma (reference interval 0.5-10.0 ng/mL) reflect the level of beta-cell function associated with insulin resistance, and indicate insulin secretory failure. Measuring C-peptide concentration in plasma provides a guide for therapy in diabetes. Because the quantitation of plasma C-peptide has been performed by an immunochemical technique, the accuracy strongly depends on that of the standard material. To establish a precise quantitation technique for blood C-peptide covering the range of the reference interval, we have developed chemical modification and immunoaffinity purification technique for the determination of serum C-peptide by isotope-dilution mass spectrometry.

Experiment

Two concentrations of serum sample were prepared by adding the certified reference material of C-peptide (NMIJ CRM 6901-a) to human serum to be 0.3 and 10 ng/mL. The isotopically labeled C-peptide, D_8-Val_2-C-peptide was used for the internal standard. A solid phase extraction was performed using Oasis HLB and MCX (Waters) , and a immunoaffinity capture was performed using a Magnosphere immobilized magnetic beads (JSR) with an anti-C-peptide antibody. AQC (6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) reagent was used for N-terminal modification of the peptide. An LC-MS/MS analysis was conducted by Prominence 20A HPLC (Shimadzu) with a reversed-phase C18 column and a TSQ Quantum Discovery tandem quadrupole mass spectrometer (Thermofisher Scientific) equipped with electrospray ion source in selected reaction monitoring (SRM) mode.

Results and Discussion

We first examined the high concentration (10 ng/mL) of serum sample by a two-step solid phase extraction using Oasis HLB and MCX prior to LC-MS/MS. The deviation of the quantitative results was more than 10 %. To improve the sensitivity and reduce the deviation, the enriched sample by the solid phase extraction was modified with AQC reagent. The AQC-modification increased 10-fold in the signal intensity and reduced the deviation on the measurement to less than 5 %. In case of the low concentration (0.3 ng/mL) of a serum sample, we could not observe the signal of the AQC-modified C-peptide using solid phase extraction for the sample enrichment. However, we successfully quantified AQC-modified C-peptide with 10 % of the deviation by an immunoaffinity purification using anti-C-peptide antibody-immobilized magnetic beads instead of the solid phase extraction prior to the AQC-modification. In conclusion, we have successfully developed the quantitation method for serum C-peptide in the range of 0.3-10 ng/mL with the deviation of 5-10 % by combination of the immunoaffinity purification and the chemical modification by AQC reagent.

Poster Session

Thursday, 20th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Session 38: Mass Spectrometry for Metabolic Diseases

PTh-098

13:30 – 14:40

Label-Free Screening for Epigenetic Drug Discovery Using Mass Spectrometry to Monitor Protein and Nucleic Acid Modification Events

Jennifer Rossi, Peter Rye, Lauren Frick, Hisashi Iwase, Masahiro Maeda, William LaMarr

Agilent Technologies

Keywords:
epigenetics, label-free, mass spectrometry, histone, methylation

Novel aspects:
This research demonstrates a novel and high-throughput means of analyzing epigenetic targets, often difficult to investigate during the drug development process.

Abstract:
Epigenetics, the study of changes in gene expression caused by mechanisms other than deviations in DNA sequence, is rapidly emerging as a field with tremendous drug development potential across a broad range of therapeutic areas. Epigenetic discovery via traditional screening technologies is complicated by the ability of these enzymes to impart multiple modification events onto singular substrates resulting in complicated and dynamic enzyme kinetic environments. In this study, we investigated the use of mass spectrometry as a universal platform for the detection and quantification of multiple protein and nucleic acid modification events (i.e. histone acetylation status, histone methylation status, DNA methylation status) . The use of mass spectrometry facilitates direct detection of native enzyme substrate and product pairs, and allows for rapid assay development of a wide array of chemical analytes (peptides, oligonucleotides, whole proteins, etc) . The label-free methodology mass spectrometry provides can enable targeted screening across a large collection of epigenetic processes and represents a ubiquitous approach to this complex research area.

PTh-099
11:10 – 12:20

Comprehensive analysis of human proteome in hippocampus tissue from Alzheimer disease patient.

hyekyeong Min[1], Hyung Joo Lee[2], Se-Young Kim[1], Gun Wook Park[1], In Jung Ji[1], Kyung-Hoon Kwon[1], Jong Shin Yoo[1], Young Mok Park[1], Jin Young Kim[1]

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Keywords:
alzheimer hippocampus proteome ETD analysis

Novel aspects:
A total of 5337 different proteins were confidently identified in control samples and 4990 in AD (alzheimer disease) patients, respectively.

Abstract:
Alzheimer's disease is a progressive neurodegenerative disorder and the most common form of dementia. The disease is confirmed by the presence of neuritic plaques and neurofibrillary tangles in the cerebral cortex at autopsy, but the accuracy of antemortem diagnosis, especially at the early stages of the disease, is not ideal. Thus, there is a substantial need for the discovery and validation of diagnostic biomarkers. We report a global proteomic analysis of hippocampus tissue from normal human and Alzheimer patient. Proteins were separated into soluble and membrane fractions. Proteins were digested with trypsin, and resulted peptides were further separated into 12 fractions by off gel isoelectric focusing method. Each samples were analyzed by UPLC coupled with reversed phase LC-ESI-MS/MS analysis and we applied both collision induced dissociation (CID) and electron-transfer dissociation (ETD) based fragmentation. Analytical column was home-made microcapillary column consisting of C18 (Aqua ; particle size 3 μm) packed into i.d. 75 μm, o.d. 360 μm silica tubing. For protein identification, the MS/MS data were analyzed using the IPI human database with the protein discoverer software. A total of 5337 different proteins were confidently identified in control samples and 4990 in AD (alzheimer disease) patients, respectively.

PTh-100
13:30 – 14:40

Rapid and sensitive determination of the intermediates of advanced glycation end products in human nail by UPLC-ESI-TOF-MS

Jun Zhe MIN[1], Makoto Yamamoto[1], Tatsuya Higashi[2], Kenichiro Todoroki[1], Koichi Inoue[1], Toshimasa Toyo'oka[1]

[1]School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan, [2]Faculty of Pharmaceutical Sciences, Tokyo University of Science, Japan

Keywords:
Human nail, 4,5-Dimethyl-1,2-phenylenediamine ; 3-Deoxyglucosone ; Methylglyoxal ; Glyoxal

Novel aspects:
As we know, for the first time, these three kinds of the dicarbonyl intermediates of AGEs, which were 3-DG, MG and GO, were first found in human nail samples.

Abstract:
The resolution of the intermediate Advanced Glycation End products (AGEs) in human nail was carried out by the combination of 4,5-dimethyl-1,2-phenylenediamine (DMPD) derivatives and ultra performance liquid chromatography with electrospray ionization time-of-flight mass spectrometry (UPLC-ESI-TOF-MS). The reaction of the reagent with 3-Deoxyglucosone (3-DG), methylglyoxal (MG) and glyoxal (GO) effectively proceeds at 60°C for 2 hr. The resulting derivatives were efficiently separated by a gradient program (a mixture of H_2O and CH_3CN containing 0.1% formic acid (HCOOH)) using a reversed-phase ACQUITY UPLC BEH C_{18} (1.7 mm, 50 mm × 2.1 mm i.d.) column and sensitively detected by TOF-MS. The detection limits (S/N= 5) of the TOF-MS were 10-50 fmol. A good linearity was achieved from the calibration curves, which was obtained by plotting the peak area ratios of the analytes relative to the internal standard (IS), i.e., 2,3-hexanedione, versus the injected amounts of 3-DG, MG and GO (r^2> 0.999), and the intra-day and inter-day assay precisions were less than 6.89 %. The derivatives of the compounds in human nail were successfully identified by the proposed procedure. As we know, for the first time, these three kinds of dicarbonyl intermediates in the formation of AGEs, which were 3-DG, MG and GO, were first found in human nail samples. Using these methods, the amounts of compounds in the nails of healthy volunteers and diabetic patients were determined. When comparing the index from the diabetic patients to those from healthy volunteers, there is no significant difference in the content of the MG and GO in the nails. However, a statistically significant (P<0.001) correlation was observed between the 3-DG concentrations. Therefore, because the proposed method provides a good mass accuracy and the trace detection of the dicarbonyl intermediates of AGEs in human nails, this analytical technique could be a noninvasive technique to assist in the diagnosis and assessment of disease activity in diabetic patients. Here we present a novel sensitive, simple method for the simultaneous determination of dicarbonyl compounds in human nails.

PTh-101 Determination of flavonoids in infected plants using LC-MS/MS

11:10 – 12:20

Jong Sung Jin[1], Hae Gyeong Kim[1], Mee Sung Lee[1], Mi Jin Kim[1], Sung Chul Shin[2]

[1]Korea Basic Science Institute, Busan, Korea, [2]Gyeongsang National University, Jinju, Korea

Keywords:
Flavonoids, LC-MS/MS, Infected plants, Metabolomics

Novel aspects:
The flavonoid content in the infected plant with the disease development decreased inithially after infection but then gradually increased before finally progressively decreasing.

Abstract:
Flavonoids play an important role as pre-infection inhibitors, providing plants with a certain degree of basic resistance against pathogenic microorganisms. It was confirmed by LC-MS/MS that as procedure of disease.
In this work we identified the flavonoids of the plants and compared their characteristic chromatographic fingerprints with infected one using liquid chromatography electrospray ionization mass spectrometry (LC-MS/MS) .

PTh-102

13:30 – 14:40

Development of methods to identify previously unknown secondary metabolites from myxobacteria based on a comprehensive metabolic profiling workflow

Thomas Hoffmann[2,3], Daniel Krug[2,3], Clive Seymour[1], Gabriela Zurek[4], Aiko Barsch[4], Rolf Mueller[2,3]

[1]Bruker BioSciences Pty. Ltd., Preston, Australia, [2]Pharmaceutical Biotechnology, Saarland University, Saarbruecken, Germany, [3]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbruecken, Germany, [4]Bruker Daltonik GmbH, Bremen, Germany

Keywords:
non-targeted metabolomics, identification of secondary metabolites, high resolution MS and MS/MS

Novel aspects:
Characterization of complex samples by combining targeted and non-targeted LC-TOF-MS based Metabolomics workflows - an efficient de-replication strategy.

Abstract:

Introduction:
The extraction of relevant information from complex data sets remains an important bottleneck in (microbial) metabolomics research. Compared to a focused, targeted approach this is even more important in non-targeted metabolomics, where the goal is the identification of all compounds produced by a particular bacterial strain. Many of these compounds are part of primary metabolism and therefore out of scope when research concentrates on secondary metabolites. Other metabolites may be very common for a certain genus of bacteria and already well known but the large subset of compounds that are "really new " is hard to identify.
By combining targeted and non-targeted metabolomic approaches, including searches against in-house and freely available data bases, it is possible to narrow down and identify numerous features derived from HPLC-high resolution MS/MS measurements.

Methods:
6 biological replicates each of myxobacterial (Sorangium cellulosum) extracts and growth medium blanks were separated by gradient elution at 600 ul/min and 45 degree C using a C18 column on an RSLC system (Dionex) . ESI-MS measurements were performed using positive ionization on a maXis QTOF (Bruker Daltonik) with a repetition rate of 2 Hz for MS and 3 Hz for MS/MS. Targeted screening was carried out using precise extracted ion chromatograms (XICs) , retention time and isotope pattern evaluation in the TargetAnalysis software . Data pre-processing, statistical interpretation and creation of a Scheduled precursor list (SPL) for targeted MS/MS experiments was performed with the ProfileAnalysis software.

Results:
The automated feature finding algorithm detected 2000 - 5000 features within one HPLC-MS chromatogram on a myxobacterial extract. In a first step these features were subjected to a search against an in-house database using accurate mass, isotope pattern fit, and retention time in order to identify known compounds. Non-targeted metabolite profiling, using statistical methods such as ANOVA, t-test as well as PCA analysis, identified 176 features related to the growth of the bacteria by comparing myxobacterial extracts to blank samples (growth medium) . These features were automatically added to a scheduled precursor list (SPL) to focus fragmentation experiments on the relevant subset of compounds within the complex mixture. This enabled fragmentation of low abundant features which might have been missed during an automatic precursor selection without predefined compounds of interest. High resolution full scan MS and MS/MS spectra were used to identify target metabolites by queries in open source libraries (e.g. METLIN) . Remaining unidentified features were subsequently compared to an in-house database to identify putative derivatives of known compounds. Calculation of sum formulae suggests the presence of new ambruticin derivatives. 63 out of 176 filtered features from myxobacterial extracts could be identified based on MS/MS data. The applied strategy effectively supported the de-replication, i.e. identification of already known compounds in complex metabolite extracts, thereby facilitating the screening for "new " bacterial secondary metabolites.

PTh-103
11:10 – 12:20

Use of proton-affinitive derivatization in HPLC-positive-ESI-MS analysis of biologically important carboxylic acids

Hajime Kato, Shin Mitsuzuka, Kowa Yamashita, Mitsuteru Numazawa

Tohoku Pharmaceutical University, Sendai, Japan

Keywords:
lipidomics, proton affinity derivatization, bile acids, eicosanoids, fatty acids

Novel aspects:
Picolyl esters as proton affinity tag for analysis of carboxylic acids using HPLC-ESI-MS were investigated.

Abstract:

Introduction

Bile acids, fatty acids and eicosanoids are biologically important as biomarker for diagnosis of various diseases or inborn error of metabolism. However, these substances generally show poor sensitivity in HPLC-ESI-MS analyses with their naked molecules. Introduction of proton-affinitive or permanently charged tags into carboxyl moieties of such molecules proved to be advantageous to improve detection sensitivity of these molecules toward ESI in HPLC-MS. During the course of our studies on the analysis of hydroxy steroids by HPLC-ESI-MS, we have found that the introduction of the proton-affinitive tags (picolinate : Pi derivative) enabled to determine estrone, estradiol, testosterone, dihydrotestosterone, dehydroepiandrosterone, pregnenolone, cortisol, corticosterone and aldosterone molecules with the limit of quantification (LOQ) of approximately 0.5-1 pg/ml human serum by selected reaction monitoring (SRM) in a positive mode. The aim of this study is to develop proton-affinitive Pi tags introducted by simple derivatization for HPLC-ESI-MS analyses of biologically important carboxylic acids.

Method

Derivatization : Carboxylic acids (bile acids, fatty acids and eicosanoids) were treated with 2-bromomethylpyridine or 2-(bromomethyl)-6-methylpyridine in CH_3CN in the presence of diisopropylethyamine. The derivatives thus obtained were purified by solid-phase extraction using ODS cartridge using 90% CH_3CN-H_2O as an eluting solvent. Identification : The structures of derivatives were confirmed by high resolution ESI-MS and ^{1}H-NMR spectroscopy. HPLC-MS : Finnigan TSQ Vantage AM (ESI-positive) ; column : X-Bridge (C18, 150 mm x 2 mm I.D., 5 mm, Waters) ; mobile phase : CH_3CN-H_2O-CH_3COOH (65 : 35 : 0.1 v/v/v) ; flow rate : 0.2 ml/min.

Preliminary data

In the case of hydroxysteroids, we selected picolinic acid and its related compounds as proton-affinity tags in our previous report. In this case, esterification of hydroxysteroids with picolinic acid was performed by mixed anhydride method based on the use of 2-methyl-6-nitrobenzoicanhydride with more than 70 % yields. In this report, we selected 2-bromomethypyridine or 2-bromomethyl-6-methylpyridine as proton-affinity tags for derivatization of carboxylic acids. In itial efforts were focused on the derivatization of bile acids as reference standards. Treatment of bile acids (cholic acid, chenodeoxylcholic acid, ursodeoxycholic acid, deoxycholic acid and lithocholic acid) with 2-bromomethylpyridine in CH_3CN solution in the presence of diisopropylethylamine gave the expected picolyl ester of carboxylic acids. The picolyl ester of each bile acid provided well-shaped HPLC peak without formation of by-products. The positive-ESI mass spectra of the picolyl ester of these bile acids were characterized by the appearance of protonated molecule as base peaks. SRM utilizing the inherent transition for each picolyl ester of bile acid enabled to determine these bile acids with extremely high sensitivity. Quantitation of these bile acids utilizing stable isotope labeled internal standards was also investigated. An application of the present derivatization method to fatty acids and some eicosanoids which include carboxyl group were also discussed.

PTh-104

13:30 – 14:40

Comprehensive CID with accurate mass measurement for analyte identification in metabolomics and natural products: HPLC-TOF-MS analysis with database searching

Joe Binkley, Binkley Joe, Kevin Siek, Li Zhang

LECO Corporation

Keywords:

Metabolomics, LC/MS, MS/MS, Databases

Novel aspects:

Comprehensive fragmentation with deconvolution and the mass accuracy and resolving power of the TOF MS enables analyte characterization in metabolomics and natural products analysis using traditional MS/MS databases.

Abstract:

Tandem mass spectrometry (MS/MS) has become the benchmark for analyte identification using mass spectrometry with the generation fragment ions occurring through varied mechanisms. Among these, collision induced dissociation is by far the most common. The other prominent tool for characterization purposes in mass spectrometry is accurate mass analysis. This has been the domain of FTMS and magnetic sector systems but time of flight systems have made significant inroads in the past decade. The growing presence of hybrid instruments which provide both MS/MS and accurate mass information has created even more opportunities. An alternative to these approaches is post source collision induced dissociation on a high performance time of flight system. When fragmentation occurs in a controlled pressure region and high resolving power and mass accuracy is combined with analyte deconvolution software which generates precursor and fragment ion spectra there is a powerful synergy. In this study post source, deconvoluted fragment ion spectra are generated for analytes detected in complex natural product samples. The spectra are searched against common databases for MS/MS spectra including Metlin and the quality of the hits and the spectra are compared. The results show that across a range of signal-to-noise levels and m/z ranges the post source fragment ion spectra provide spectra comparable to library spectra and high integrity hits. Much of this is attributable to the high mass accuracy observed in both the precursor and product on spectra (>45,000 resolving power and better than 2 ppm mass accuracy) . In addition, the post source dissociation provides superior relative isotope abundance measurements for precursor and product ions which facilitates analyte identification. Specific examples of the utiilty of this approach in metabolomics and natural product characterization are discussed.

PTh-105
11:10 – 12:20

Acylcarnitine analysis by ESI-MS/MS with smaller amount of sample and rapid analytical time

Hironori Kobayashi[1], Yuichi Mushimoto[1], Yuki Hasegawa[1], Kenji Yamada[1], Jamiyan Purevsuren[1], Tomoo Takahashi[1], Toshikazu Minohata[2], Junko Iida[2], Yamaguchi Seiji[1]

[1]Shimane University, Izumo, Japan, [2]Shimadzu Corporation, Kyoto, Japan

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Keywords:
newborn screening, acylcarnitine, amino acids, rapid analysis

Novel aspects:
Rapid and small-volume-injection analysis of amino acids and acylcarnitines by ESI-MS/MS

Abstract:

Introduction

After late 1990's, analysis of acylcarnitines and amino acids by MS/MS has become popular in newborn screening. This screening made it possible to examine more than 20 diseases at once including disorders of amino acid metabolism, organic acidemia, and fatty acid oxidation disorders. Currently, a non-derivatization method is predominant. Current method of analysis requires 5 -20 ml of injection volume, and requires over two minutes at one analysis, approximately 72,000 samples a year. Because a sample is extracted from dried blood spot on filter paper in newborn screening, smaller volume of injection is preferred for less maintenance. We developed the method of more rapid and smaller injection volume by using Nexera MP and LCMS-8030 triple quadrupole mass spectrometer (ShimadzuCorporation) .

Methods

A 3 mm punch of blood spot was processed using a NeoBase Non-derivatized MSMS Kit (PerkinElmer) and analyzed by multiple reaction monitoring (MRM) . Dried blood spot of known concentration in each analyte were used. Samples were measured by Nexera MP and LCMS-8030 equipped with an ESI source positively charged. Flow rates of mobile phase provided in Kit was 0.1 ml/min. Injection volume was changed in 10 µl, 5 µl, 2 µl, and 1 µl, respectively.

Results

Sensitivity was sufficient even in 1 µl of injection volume, as compared with original injection volume of 10 ml. Accuracies and precision in intra- and inter-day assay were within 10%. An analysis cycle of 74 seconds per sample was achieved in this situation.

Conclusion

Combination of Nexera MP and LCMS-8030 achieved higher throughput, more than 100,000 samples a year, than current screening.

PTh-106
13:30 – 14:40

Simultaneous determination of acylcarnitines and amino acids for inborn error of metabolism using UPLC/MS/MS

Haruka Oda[1], Yasuhiro Maeda[1], Tetsuya Ito[1], Yuji Hotta[1], Yoko Nakajima[1], Sayaka Kato[1], Shinji Saito[1], Naruji Sugiyama[2], Kazunori Kimura[1]

[1]Nagoya City University, Nagoya, Japan, [2]Aichi-Gakuin University, Nagoya Japan

Keywords:
acylcarnitine, newborn screening, amino acids, UPLC MS/MS, metabolic disease

Novel aspects:
We developed detail determinaton method of acylcarnitines and amino acids without delivatization for metabolic disease by UPLC/MS/MS. This method enables acute diagnosis of metabolic disease.

Abstract:
Background: Tandem mass spectrometry (MS/MS) analysis of acylcarnitines and amino acids has been used to screen newborns for organic acidemias, fatty acid oxidation defects and amino acid metabolism abnormality. Although this method provides results in a few minutes, the method cannot separate acylcarnitine isomers and amino acid isomers. Moreover, the peak intensity of analyte by MS/MS would be unstable by ion suppression to detect many kinds of acylcarnitines and amino acids at the same retention time in the chromatography. We previously reported determination method of acylcarnitine isomers from free carnitine (C0) to octanoylcarnitine (C8) by HPLC/MS/MS. Simultaneous analysis of both short-chain acylcarnitine and long-chain acylcarnitine by HPLC was very difficult as the polarity of these acylcarnitines is different greatly. The determination method of C0 to octadecanoylcarnitine (C18) was developed by derivatizing acylcarnitines. However, the derivatization reagent is atypical and derivatization reaction takes trouble. We investigated the simultaneous determination method of acylcarnitines (C0-C18) and amino acids (Val, Leu, Ile, Met, Phe, Orn, Cit, Arg, Tyr) without derivatization by ultra high performance liquid chromatography (UPLC) -MS/MS. A more accurate diagnosis of inborn error of metabolism is enabled by this analytical method.
Method: A mixture of 5 micro L of 28 acylcarnitines, 9 amino acids, corresponding labeled acylcarnitines and labeled amino acids was injected onto Acquity UPLC BEH C18 column (2.1 x 150 mm, Waters) and eluted at a flow rate of 0.3 mL/min using a step gradient alternating between 0.08 % aqueous ion-pairing reagent (IPCC-MS3, GL sciences) and 0.3 % formic acid in methanol. Acylcarnitines and amino acids were analyzed in positive ion multiple reaction monitoring (MRM) mode of Quattro Premier XE triple quadrupole mass spectrometer (Waters) .
Acylcarnitnes and amino acids were extracted from serum of patients with inborn error of metabolism using an OASIS MCX solid phase extraction cartridge (Waters) . The extracted solution was analyzed by above method.
Results: All acylcarnitine isomers were separated in 35 min and all amino acids were analyzed in 13 min using UPLC. The limits of quantifications (LOQs) were 0.05 micro mol/L for acylcarnitines and 1.0 micro mol/L for amino acid, respectively. The precision data for serum sample showed that intra-day coefficients of variance (CVs) at three concentrations were less than 15 %. Acylcarnitines and amino acids which were not the marker in screening were specifically detected in analysis of the serum samples of patients with inborn error of metabolism. For example, methylmalonylcarnitne (C4DC) and 3-methylcrotonylcarnitine (C5 : 1) were accumulated in the serum of patients with methylmalonic acidemia (marker ; propionylcarnitine (C3)) and holocarboxylase synthetase deficiency (marker ; 3-hydroxyisovalerylcarnitine (C5OH)) , respectively.
Discussion: We developed simultaneous determination method for acylcarnitines (C0-C18) and amino acids without derivatization. The analytical time is shorter by using UPLC and the quantification of low concentration is possible because analysis in MRM mode is very sensitive. The separation of analyte by UPLC column would achieve stability of the individual peak sensitivity by MS/MS. Therefore, concentration of near the cut-off value is determined precisely and the decrease of the pseudo positive is expected. The acylcarnitines and amino acids profile obtained by this method enabled a correct diagnosis. This method can apply to monitoring of the effect of treatment by observing a change of acylcarnitines and amino acids.

PTh-107

11:10 – 12:20

An Attempt to Quantitative Analysis for Clinical Proteomics by Nano LC-nano-ESI-SRM-MS Using Stable Isotope-labeled Iodoacetanilide as well as N-Ethylmaleimide

Sadamu Kurono[1,2], Yuka Kaneko[1,2], Shuji Matsuura[1], Satomi Niwayama[3]

[1]Osaka University, Osaka, Japan, [2]Wako Pure Chemical Industries, Ltd., Osaka, Japan, [3]Texas Tech University, Lubbock, TX

Keywords:
Quantification, Stable isotope, Iodoacetanilide, N-ethylmaleimide

Novel aspects:
A combination of stable isotope-labeled and -unlabeled iodoacetanilides or N-ethylmaleimides and LC-ESI-SRM-MS offers a new method of absolute quantification for clinical proteomics.

Abstract:
Quantitative analysis of proteins is essential to clinical proteomics. We have developed a methodology for relative quantification of proteins by modifying cysteine residues with stable isotope (^{13}C) -labeled iodoacetanilide ($^{13}C_7$-IAA) or -unlabeled iodoacetanilide (IAA) as well as stable isotope (d) -labeled *N*-ethylmaleimide (d_5-NEM) or -unlabeled *N*-ethylmaleimide (NEM), followed by nano LC-nano-ESI-mass spectrometry. In addition to the combination of the above labeling, the selected reaction monitoring (SRM) technique, accomplished by specifying the precursor ion and specifically monitoring its few most intense and most reliable CID product ions, was also applied to identification and absolute quantification by the above mass spectrometer for clinical proteomics. We examined the possibility for absolute quantification of commercial proteins such as bovine serum albumin (BSA) and some candidate proteins for breast cancer biomarkers from nipple discharge of breast cancer patients.

For absolute quantification of BSA, we selected two tryptic peptides for SRM and obtained their synthetic peptides. The synthetic peptide solution adjusted at around pH 9.0 or 7.0 and reduced with dithiothreitol or TCEP (Bond-Breaker TCEP Solution, Neutral pH, Thermo Fisher Scientific) for IAA/$^{13}C_7$-IAA or NEM/d_5-NEM modification, as the case may be, was divided into two solutions. One of the solutions was incubated with an excess amount of 100 mM IAA DMSO or NEM solution, while the other with 100 mM $^{13}C_7$-IAA DMSO or d_5-NEM solution. The IAA- or NEM-modified solution was adjusted to the following five concentrations : 1000, 500, 100, 50, and 10 fmol/μL, while the $^{13}C_7$-IAA- or d_5-NEM-modified solution was adjusted to 100 fmol/μL. Each IAA- or NEM-modified solution mixed with $^{13}C_7$-IAA- or d_5-NEM-modified solution was loaded into nano LC (UltiMate 3000, Thermo Scientific DIONEX) -nano-ESI-Ion Trap MS (HCTultra, Bruker Daltonics) system and a standard curve was created by the SRM technique.

For absolute quantification of commercial proteins we used three kinds of proteins : BSA, ovalbumin, and α-lactalbumin. In the case of BSA, we selected two tryptic peptides for the SRM analysis, which were observed at a high intensity on MS. Two clusters of peaks representing IAA- and $^{13}C_7$-IAA-modified tryptic peptides were observed to be 7 Da apart due to the presence of seven ^{13}C atoms and detected with the same retention time, which means that the isotope effects by the introduction of IAA and $^{13}C_7$-IAA do not exist in this system. One of the above two peptides, LCVLHEK, was custom-synthesized, and modified with IAA or $^{13}C_7$-IAA. Five IAA-modified peptide solutions with different concentration (1000, 500, 100, 50, and 10 fmol/μL) were prepared, and each solution was mixed with a constant concentration of $^{13}C_7$-IAA-modified peptide (100 fmol/μL). The SRM conditions were as follows : IAA-modified LCVLHEK (*m/z* 487.8 doubly-charged precursor ion >*m/z* 333.2, 625.4 singly-charged product ions), $^{13}C_7$-IAA-modified LCVLHEK (*m/z* 491.3 (same as above) >*m/z* 340.2, 625.4 (same as above)), and the chromatogram area ratio of *m/z* 333.2/340.2 (Cys-containing) and *m/z* 625.4/625.4 (non-Cys-containing). The standard curve based on the results of above five mixtures had R^2 value that was close to 1 (0.995). The relative standard deviation (RSD) values indicated repeatability were calculated from three replicated SRM analyses for each mixture and the RSD ranged from 3.9 % to 17.0 %. The R^2 values obtained from other commercial proteins also showed those close to 1, and the RSD values were similar to those of BSA. The similar result was also obtained using NEM/d_5-NEM and from other two proteins. Therefore the combination of the IAA and $^{13}C_7$-IAA modification in addition to SRM was found to be an effective tool for absolute quantification for proteins. Furthermore we are also trying to apply this technique to absolute quantification of some candidate proteins for breast cancer biomarker.

PTh-108 Multivariate analysis software to support large scale MRM study

13:30 – 14:40

Atsushi Ogiwara[1], Hisae Anyoji[1], Jun Watanabe[2], Junko Iida[2], Mitsuhiro Kanazawa[1]

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Keywords:
MRM, Multivariate Analysis, Alignment, Quantification, Qualification

Novel aspects:
Alignment functionality in Signpost MS software improves the capability of quantification and qualification of multi-sample MRM data.

Abstract:
MRM analysis is widely used in various areas including metabolomics for the sake of quantification and qualification. In order to carry out such analyses, a number of MRM transitions for a large number of samples must be processed. Software tools have been desired to treat such data with user-friendly interfaces to conduct from data capturing to multivariate analysis. We have developed integrated multivariate analyses software named Signpost™ MS, which can treat various kinds of MS instruments data. Previously, this software is mainly used for the analyses of scan data, where data are represented as a series of spectra. Here we have expanded the functionality of the software to support MRM data.

One of the important functions of this software is "alignment ". In the case of scan data derived from LC-MS, there are three fundamental axes : 1) separation axis as retention time, 2) spectrum axis as m/z, and 3) quantity axis as ion intensity. Though mass values are quite solid, retention time by liquid chromatography fluctuates and time axes should be aligned between measurements. Once time axes are aligned, now corresponding peaks in different measurement can be observed at the same position of the aligned time axis.

In the case of MRM data, where the dimension of spectrum axis is reduced to single or several transitions, still we provide the alignment function to overlay peak shapes by adjusting time axes.

When we measure an analyte of high concentration, it might not be difficult to detect the peak derived from the analyte. However the lower the analyte concentration, the peak height becomes the lower, and the detection of the peak becomes the more difficult. In such a case, we cannot use intensity to detect peaks. However, if we can predict the location of the peak on time axis, we can detect the peak.

Alignment function is also useful in another case where several candidate peaks can be observed due to interference peaks or noise. In such a case, location information of time axis may be a decisive factor.

Alignment is conducted based on the similarity of shapes of chromatogram. Thus it would be hard to align chromatograms of poor shapes, due to the weak signal response of the analyte. However, in such cases we can use other transition information that is measured simultaneously. For example, internal standard signals can be used for quantification measurement, and other analyte transition signals can be used for MRM profiling.

Once we have capture peaks, then they will be presented visually to validate authenticity of the selected results. We also introduce some basic rules to evaluate the validity of peak shapes.

The software begins by loading raw data from an LC-MS experiment. In the case of MRM assay, loaded data are rearranged according to transitions. Grouping information of samples should be given by users. For samples belonging to the same sample group, time axes are aligned each other by using common transition data. Then peaks are detected and presented visually for validation. Results of the peak shape validity evaluate on are also presented. Conformed peaks are arranged into a table to be analyzed by multivariate analyses like PCA or cluster analyses.

In this study, we show novel use of MRM data in the context of metabolomics biomarker discovery using an LCMS-8030 triple quadrupole mass spectrometer integrated with Nexera UHPLC system (*Shimadzu Corporation, Kyoto, Japan*) .

PTh-109
11:10 – 12:20

High Pressure Acid Dissolution of alpha-alumna for trace elements determination by ICP-MS

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Keywords:
alpha-alumna, high pressure acid dissolution, ICP-MS, JCRM R 031, metal impurities

Novel aspects:
This presentation describes alpha-alumna sample can be easily dissolved by simple acid treatment

Abstract:
High purity α-alumna has important uses in the electronics and ceramics industries. The properties of α-alumna are much affected by the level of impurities present, and hence the determination of trace elements in this material is of great practical importance. It is well known that the dissolution of α-alumna sample for analysis of trace impurities is very difficult.
This presentation describes α-alumna sample can be easily dissolved by simple acid treatment. Granule and wafer type of α-alumina sample were pulverized with vibratory micro mill device (FRITSCH, GmHB) . And then, an enough amount of α-alumina powder sample (1 g) was decomposed with 5 mL of H_2SO_4 and 15 mL of deionized water in 50 mL size of Teflon vessel of high pressure acid digestion device by heating in drying oven for 16 h at 230° C. After cooling, the decomposed sample was diluted with deionized water and then metal impurities were analyzed by ICP-MS.
The elements studied include Co, Cu, Cr, Ga, Fe, Mg, Mn, Na, Si, and Zn. The limit of Quantification (LOQ) for each element in ICP-MS was presented.
For the method validation, the metal impurities in the certified reference material of alumina (JCRM R 031, 032, 033) were analyzed by the same method described in this paper and compared with certified values.

PTh-110
13:30 – 14:40

High-Speed Survey Method for Photo-degradation Products of Pharmaceuticals Using UV-LED Lighting Device and DART-TOF Mass Spectrometer

Toshio Tashima[1], Kaori Asano[2], Takeyuki Suzuki[2], Tsuyoshi Matsuzaki[2], Takeshi Nakano[3]

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Keywords:
PPCPs, Photo-degradation, DART-MS, UV-LED, Pharmaceuticals

Novel aspects:
This highest-speed and simplest method will become extremely useful to survey photodegradation products of pharmaceuticals and personal care products (PPCPs) in environment and drug-development research fields.

Abstract:
Over the past decade, attention as organic pollutants in aquatic environments has been gradually increasing to pharmaceuticals and personal care products (PPCPs) . A large amount of pharmaceuticals have been released continuously into aquatic environments for more than a century. Thus, there have been many reports about the occurrence of pharmaceutical products in river water, seawater, and wastewater.

GC-MS and LC-MS or LC-MS/MS are used as the most common techniques for determination of pharmaceuticals in aquatic environments. The selected ion monitoring (SIM) , the selected reaction monitoring (SRM) and the multiple reaction monitoring (MRM) methods are utilized as the technique for determination of pharmaceuticals in aquatic environment. When these techniques are used for determination of pharmaceuticals, it is required to obtain the information about the specific ions of target pharmaceuticals prior to starting analyses of them. This indicates that these convenient techniques become useless entirely in determination of unknown compounds.

Photo-degradation is suggested to play an important role in elimination of some of PPCPs from surface waters. In fact, the fate of pharmaceuticals has been recognized by solar and ultraviolet irradiation to them. However, there seems to be also a few reports on the determination of photo-degradation products of pharmaceuticals in aquatic environments in spite of taking a large variety of pharmaceuticals.

Most of investigators have performed the photo-degradation studies by sunlight and/or UV radiation using an appropriately devices equipped with a mercury lamp (Hg-vapor lamp) or a xenon lamp (Xe lamp) . The radiation intensity is ca. 300 W/m^2 (daily average) in the case of solar light[1)] and 400 800 W/m^2 in the cases of Hg-vapor lamp[2)] or Xe lamp[3)] which consume a relatively long radiation time (about 10 hours) to obtain the significant fate of pharmaceuticals. In addition, these radiation conditions require some cooling system to prevent heat affection. If the photo-degradation study can be performed faster and easier, it will become to assess the photo-degradation products readily. Therefore, we developed the very simple and fast method using a UV-LED lighting device and a DART-TOF mass spectrometer[4)].

A small size UV-LED lighting device has been available recently for UV cure processing on pinpoint. At present time, there are several devices giving ultra violet light (λ max : 365 nm) and high power radiation intensity (to 9500 mW/cm^2) with no heat affection. We used the cheap model with radiation intensity of ca. 4000 mW/cm^2 (40000 W/m^2) and beam diameter of 4 mm (ULEDN-101, NS-Lighting) . After preparation of sample solution using methanol and/or acetonitrile (ca. 5 mg/ml) , a portion of several micro-L was applied on the reverse-phase type of TLC plate (RP-2 F_{254}, Merck) using a glass capillary tube and UV light was irradiated for appropriate time (within 120 sec) on the sample spot (diameter : ca. 3 mm) . DART-Mass spectrometry was utilized to detect the photo-degradation products on the TLC plate, because the DART ionization provides direct analysis under atmospheric pressure and few fragmentation of molecular ion. In this study, we used DART-SVP100 (IonSence) for ionization of samples and Accu-TOF mass spectrometer (JMS-T100LC, JEOL) for acquisition of mass spectral data.

In this conference, we will present our high advantage method for exploring photo-degradation products of pharmaceuticals by showing the results in the cases of Ibuprofen, Naproxen, Ketoprofen, Indomethacin and Diclofenac which are generally used as a non-steroidal anti-inflammatory drugs (NSAID) .

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PTh-111
11:10 – 12:20

Hydrophilic-interaction liquid chromatography (HILIC)-tandem mass spectrometry for quantification of oseltamivir and zanamivir in surface water and sediment samples

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Keywords:
Influenza, oseltamivir, zanamivir, river-water, sediment

Novel aspects:
Novel SPE and HILIC-MS/MS methods are developed for extraction and quantitation of oseltamivir and zanamivir simultaneously in environmental waters. Oseltamivir is detected in sediment samples for the first time.

Abstract:
Oseltamivir and zanamivir are the most prescribed antiviral drugs against influenza in Japan. Oseltamivir and its metabolite have been frequently detected in surface water bodies in Japan during the last few years particularly in seasonal influenza period posing the threat of emergence of drug-resistant genes in human pathogens. Although zanamivir is placed in the third position in terms of its use as an antiviral drug in Japan, its occurrence and fate in environmental waters are not known until now. This situation may be partially attributed to the lack of a suitable sample treatment and analysis methods for the drug. Therefore, suitable and sensitive sample pretreatment and analysis methods are highly desirable for quantitation of the drug in environmental waters. Furthermore, optimized pretreatment and analysis methods for both oseltamivir and zanamivir would be the best choice for their precise and rapid quantitation in water samples.

This article discusses the occurrence of oseltamivir phosphate (OP) , oseltamivir carboxylate (OC) and zanamivir hydrate (ZH) in water and sediment samples from Neya River in Osaka using a recently developed novel solid-phase extraction (SPE) method for simultaneous recovery of the compounds and hydrophilic-interaction liquid chromatography (HILIC) -tandem mass spectrometry (MS/MS) method for quantitation. Water and top sediment samples at three representative points (ST-1, ST-2 and ST-3) from the top towards the mouth along Neya River were taken during the 2011/2012 seasonal influenza period. The ST-1 is characterized by very small river flow and relatively bigger size sand particles in sediment. The ST-2 is characterized by drastically large river flow due to wastewater treatment plant discharge in the upstream side and medium sand particles in sediment. The ST-3 is characterized by very large river flow and silt particles in sediment.

The OP, OC and ZH were not found in both water and sediment at ST-1. They were present in water, but not in sediment at ST-2. The scenario was completely different for ST-3. The three compounds were found in water samples, while OP only was present in the sediment at ST-3. Thus, the compounds were found in abundance in water phase, while OP alone was detected in the sediment from Neya River. Batch adsorption test results for the compounds in ultrapure water with river sediment showed the highest adsorbed fraction (24%) for OP, while the values for OC and ZH were 3 % and 1 % respectively. Moreover, the fractions in water phase for OC and ZH were about 97% and 93% respectively. The value for OP was only 6 %. These results clearly indicated the highest adsorption affinity of OP to sediment, while the affinities for OC and ZH were negligibly small. It may be evident from these results that OP is more likely to be adsorbed to sediments than OC and ZH.

A newly developed SPE method for simultaneous recovery of oseltamivir and zanamivir together with a hydrophilic-interaction liquid chromatography (HILIC) -tandem mass spectrometry method were employed for accurate quantitation of the drugs in river water and sediment samples. The results demonstrated abundance of OP and OC in river water during 2011/2012 seasonal influenza period, while OP only exhibited high potential for adsorption to sediment. It became evident that monitoring the drugs, particularly OP, in water phase alone is not enough in assessing the threat of emergence of drug-resistant genes in human pathogens.

PTh-112
13:30 – 14:40

Determination of chlorinated/brominated polycyclic aromatic hydrocarbons (Cl/BrPAHs) in flue gas and ash from waste incinerator

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Keywords:
halogenated PAHs, organohalogen compound, by-products

Novel aspects:
Gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS) was applied for the comprehensive analysis of ClPAHs and BrPAHs congeners including unidentified congeners.

Abstract:
Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) such as chlorobenz [*a*] anthracene (ClBaA) and chlorobenzo [*a*] pyrene (ClBaP) have received worldwide attention because of their environmental persistence and widespread distribution. Horii et al. have showed that several ClPAHs and BrPAHs elicit dioxin-like activity with potencies comparable to those of several mono-ortho polychlorinated biphenyl (PCB) congeners. Recent reports have showed the occurrence of ClPAHs and BrPAHs in flue gas and fly ash from municipal and industrial waste incinerators. However, little is known about congener profiles and distributions of ClPAHs and BrPAHs in flue gas, fly ash, and bottom ash from waste incinerators, due to the lack of individual analytical standards. Several tens of individual ClPAHs and BrPAHs were therefore synthesized in our laboratory. In this study, we measured individual concentrations of 26 ClPAHs and 15 BrPAHs in flue gas, fly ash, and bottom ash from 8 waste incinerators. In addition, gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS) was applied for the comprehensive analysis of ClPAHs and BrPAHs congeners including unidentified congeners. Results showed that monochloropyrene (1-ClPyr) and 6-ClBaP were the dominant compounds in flue gas samples. The profiles of halogenated PAHs were similar to the profiles reported previously for urban air. Concentrations of chlorinated phenanthren and pyrene in flue gas were significantly correlated with the corresponding parent PAH concentrations. Significant correlation between ΣClPAH and ΣPAH concentrations suggests that direct chlorination of parent PAHs is the mechanism of formation of ClPAHs during incineration of wastes. Furthermore, highly chlorinated PAHs and brominated/chlorinated PAHs were found in samples by the comprehensive analysis using GC-HRTOF-MS.

PTh-113

11:10 – 12:20

SELECTIVE EXTRACTION OF ORGANOHALOGENS FROM GCxGC-HRTofMS DATA FOR GLOBAL ANALYSIS OF ENVIRONMENTAL AND BIOLOGICAL SAMPLES

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Keywords:
direct-sample-introduction, thermal-desorption, exact-mass, POPs, unknown-chemicals

Novel aspects:
It is new to extract compounds on demand from a complehensive GCxGC-HRTofMS data that includes huge number of chemicals whiout pre-setting targets.

Abstract:
There are various chemicals around us. Some of them cause problems such as environmental pollution or have adverse effects on organisms. As the number of undesired chemicals grows, they become increasingly expensive in terms of time and cost to monitor and count. This can be partly attributed to problems with analytical methods, which require a lot of time and resources, and where a skilled user is needed to measure trace levels of compounds precisely in the presence of a large amount of interference, and because the methods are individually optimized for each target substance. We are developing and improving methods and tools to overcome the problems related to the techniques used for analyzing organic environmental pollutants. We are currently developing a new apparatus consisting of a comprehensive two-dimensional gas chromatograph (GC×GC) , which is a high performance technique for the separation of chemical compounds, directly coupled with a quadrapole type tandem mass spectrometer (MS/MS) and a high resolution time-of-flight mass spectrometer (HRTofMS) . We have shown that halogenated compounds can be detected comprehensively and selectively in environmental samples by employing a neutral loss scan (NLS) on a GC×GC-MS/MS. However, the sensitivity of the NLS on this instrument was insufficient to detect trace levels of organohalogens and incapable of identifying compounds due to their unit-mass records.
Here, we report our attempts at the selective extraction of a subset from the GC×GC-HRTofMS data in order to detect and identify trace levels of organohalogens. The data was obtained by measuring several environmental and biological samples, namely flyash, soil, sediment, the atmosphere and human urine. For global analysis, some samples were measured without any purification using a 6890GC (Agilent Technologies) with a KT2004 GC×GC system (Zoex) coupled with a JMS-T100GC (JEOL) HRTofMS. A column pair consisting of 5% phenyl/phenyl-methyl silicone and 50% phenyl/phenyl-methyl silicone was employed as the liquid phase of the GC capillary columns for measurements with the GC×GC system.
Prior to data analysis, 5-15 gigabytes (GB) of raw data from the HRTofMS were converted into 0.5-2.5 GB of data in the netCDF (AIA) format. We attempted to extract only the mass spectra of organochlorines or organobromines using the several methods that we developed.
As a result, after data analysis under various mass resolution conditions, we achieved the selective extraction of the mass spectra of chlorinated or brominated compounds under a high mass resolution condition that exceeded approximately 10,000 for some samples. In contrast, low mass resolution below 1,000 was unable to extract the organohalogen data effectively. The results of this study show that data obtained with a high-resolution time-of-flight mass spectrometer are valuable for the global analysis of organohalogens, and probably of other compounds if specific data extraction methods can be devised. However, to improve the data extraction performance the deconvolution of peaks and mass spectra is required because many compounds are co-eluted even when using a GC×GC depending on the sample matrix.

PTh-114
13:30 – 14:40

Electrospray mass spectrometric observation of the interaction between environmental pollutants and biologic compounds

Hiroshi Moriwaki, Takahiro Nakagawa

Shinshu University

Keywords:
electrospray ionization ; interaction ; zinc pyrithione

Novel aspects:
Solutions containing amino acids and environmental pollutants were measured by ESI-MS for the purpose of gaining information on the behavior of the pollutants with amino acids and peptides.

Abstract:
Abstract
Interactions between biologic compounds and environment pollutants often cause various toxicities, such as defective development. Therefore, it is very important to understand the interactions.

Electrospray ionization mass spectrometry (ESI-MS) is a very powerful method for the characterization and identification of interactions between polar species, because the technique is a very soft ionization method. The technique has been frequently used for the analysis of the interactions between biological materials and chemicals (H. Moriwaki, 2002) or metal ions (H. Moriwaki, 2003) .

However, it is very difficult to research the interaction between proteins and substances by ESI-MS, because there are many complex binding sites and forms between proteins and substances. There are several examples where an investigation of a solution of a target substance with amino acids by ESI-MS was studied in order to initially serve as a simple model for complex interactions of the substance in proteins (C. L. Gatlin and F. Tureek, 2000) .

In this presentation, solutions containing amino acids and environmental pollutants were measured by ESI-MS for the purpose of gaining information on the behavior of the pollutants with amino acids and peptides. Zinc pyrithione (ZnPT) and toxic metal ions were selected as targets of this study (H. Moriwaki et al., 2009) .

Zinc pyrithione (ZnPT) is the zinc chelate of 2-pyridinethiol-1-oxide. This compound is widely used as a bactericide, fungicide and algicide in various products, such as antidandruff shampoos. A few studies have examined the toxicity of ZnPT on aquatic organisms, and it has been clarified that ZnPT can be potentially highly toxic (K. Goka, 1999) . Therefore, it is important to understand the influence of the compound on wild life and the mechanisms of the toxicity of ZnPT in order to determine the appropriate use of ZnPT. The ZnPT complex ($[ZnPT\text{-}ligand + Amino\ acid]^+$) , in which the ligand of ZnPT was exchanged by the amino acid, was detected in ZnPT solutions mixed with one of 20 amino acids by ESI-MS. Histidine and cysteine, in particular, showed a high reactivity with ZnPT, while serine and glycine showed a low reactivity.

In addition, electrospray mass spectrometric observation of the interaction of toxic metal ions with amino acids has been also studied in the same manner as ZnPT.

The procedure described in this study is very simple and suggested only a simple model for the reaction of biological materials with the pollutants. However, it provides significant information for estimating the behavior of the pollutants within the living body.

PTh-115
11:10 – 12:20

Characterization of organic pollutants in River Water by GC/MS ion profiles

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Keywords:
River pollutants, ion profiles, GC/MS

Novel aspects:
The families of compounds are shown by ion chromatograms obtained on characteristic ions. Their origin is discussed in correlation with the compounds known as molecular markers.

Abstract:
Background. The specific human activities lead to contamination of the aquatic environment with a wide variety of natural and synthetic compounds not found prior to modern times. Due to an incomplete elimination in wastewater treatment plant (WWTP) residue of contaminants are found both in waste and surface waters. Many of these compounds provide a means of identification sources of inputs and pathways of movement of chemicals through ecosystem.
In respect to this, one of priority tasks is to determine the environmental distribution of organic pollutants in the surface waters. Both anthropogenic and naturally occurring compounds are found mixed together in recent environmental samples and several of these compounds may be used as tracers to study natural processes affecting the fate and effects of chemical contaminants in water.
Objective. In the south-eastern of Europe there is little information on river water status[1]. The existent data refers at some metal trace in very critical sites, general quality studies by analyzing the benthic macrofauna but information on anthropogenic organic pollutants as individual compounds is limited to only few emerging contaminants[2]. Therefore a systematic structural investigation of individual molecular pollutant present in environmental water at regional level is crucial.
The present paper purpose is the Mass Spectrometric (MS) characterization of organic pollutants to evaluate the degree of contamination of the river waters in high populated catchments from South-Eastern of Europe. The samples were collected from few places along of the Prut River, Border River between Romania and Republic of Moldova. In the Prut Basin live more than 1000 000 inhabitants.
Methods. The samples were collected by three techniques : a) grab sample, b) using composite sample devices and c) by passive samples devices.The GC/MS analyses were performed using a Thermo Electron Polaris Q mass spectrometer operated in EI mode to 70 eV. The gas chromatograph was equipped with a capillary column HP- 5 MS (30x0.25mm) with 0.25film thickness. The temperature was programmed from 90^0C to 315^0C.
Results. The families of pollutants was visualised by chromatograms on diagnostic ions (base ion or ion of high intensity and molecular ion)[3]. A systematic study of individual compound as structure and quantity in region of border Romania-Moldavia was done based on experimental data on a big number of samples. Also was established the environmental molecular markers as identification of source pollution. The molecular marker compounds will be used in the subsequent monitoring activities to obtain the complete image of the specific pollutantsin the Prut Basin in different seasons. An number of 67 of compounds were identified. The quantity of pollutants was calculated using isotopic labeled compounds. The structure and quantity of detected compounds are discussed in relation to sampling methods and collection places. The obtained profiles of pollutants are a function of their sources and reflect the industrial and domestic activities at regional level.

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PTh-116
13:30 – 14:40

A Sensitive measurement of sucralose and acesulfame in inland and offshore waters around Japan by LC/MS and LC/MS/MS.

Shigeru Suzuki[1], Atsuko Hasegawa[2], Rina HIguchi[1], Takahiro Yamaguchi[1], Hisato Nishikawa[1], Takuhei Miyaki[1], Chiaki Oshima[1], Ryosuke Otsubo[1]

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Keywords:
sucralose, acesulfame K, environmental water

Novel aspects:
comprehensive research of sucralose and acesulfame K in environmental waters in and around Japan island

Abstract:
Introduction : Sucralose and acesulfame K are widely used artificial sweeteners, which are not likely to undergo a chemical transformation through not only natural processes but also biological reactors. Because of increasing consumption of sucralose and acesulfame K, the environmental behavior and the fate of the persistent sweeteners have been a concern. But the data are mostly limited to Europe, in particular northern Europe, and few other areas. Here we report the distribution of the artificial sweeteners in water in and around Japan islands.

Method : River water samples were collected in areas around Lake Biwa and Lake Suwa. Seawater samples between northern Japan and Sakhalin were collected by auto sampling device equipped in a cursing ship. Sample water was prepared around pH 3 for collecting sucralose (log Kow 0.78) by solid phase extraction (SPE) . For collecting acesulfame (log Kow -2.3) by SPE, dihexylammoniumacetate (DHAA) was added to the water sample neutralized with ammonium hydroxide. The river water samples were measured by LC/MS using dihexylammoniumacetate as an ion pair reagent in HPLC, and seawater were measured by LC/MS/MS with triethylamine in mobile phase.

Results : Recovery efficiencies of sucralose and acesulfame from river water fortified with the sweeteners (every 5 ng/mL) were 108% on average (RSD 2.4%) and 109% (RSD 6.8%) , respectively. Those of seawater fortified with the sweeteners (every 0.1 ng/mL) were 103% on average (RSD 11%) for sucralose and 91% (RSD 5.1%) for acesulfame.

River waters were sampled around Lake Biwa and Lake Suwa. The concentrations of sucralose and acesulfame in the 25 rivers around Lake Biwa were 1 - 960 ng/L (120 ng/L on average) and 18 - 1500 ng/L (220 ng/L on average) , respectively. The river of which the sweeteners' concentrations were higher were tended to be higher in the biochemical pollution indexes such as TOC, BOD, COD, total-N, total-P. The concentrations of sucralose and acesulfame in the 5 rivers around Lake Suwa were 0.1 0.59 ng/L (2.4 ng/L on average) and 2.0 3.1 ng/L (0.29 ng/L on average) .

Seawater was sampled at 30 sites with GPS data, time and general water qualities. The concentrations of sucralose and acesulfame were 0.2 to 42 ng/L (3.0 ng/L on average) and 0.6 to 69 ng/L (9.0 ng/L on average) , respectively. The concentrations in seawater were almost one hundredth of those in river waters. Concentration ratios of sucralose to acesulfame in seawater were varied from 0.01 : 1 to 11 : 1, which were almost similar to those in river waters (from 0.01 : 1 to13 : 1) . It is suggested that the concentrations between the two sweeteners had almost no relation to each other both in sea water and river water, and the behaviors of the two sweeteners might be different in environmental waters.

Acknowledgements : This research was kindly supported by the grant from the Sumitomo Foundation. The seawater samples were collected by Dr. Kunugi of Tokyo University of Science, highly sensitive LC-MS-MSLC/MS/MS measurements were taken by Mr. Takeda of AB Sciex Japan. The authors sincerely appreciate their contributions.

PTh-117
11:10 – 12:20

Real-time ambient air monitoring using selected ion flow tube-mass spectrometry (SIFT-MS)

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Keywords:
SIFT-MS Air-Analysis Pollutants VOC Real-time

Novel aspects:
Real-time monitoring (without sample preparation) for volatile organic compounds in the vicinity of an elementary school over an extended period.

Abstract:
Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a real-time analytical technique that detects volatile organic compounds and certain inorganic gases down to part-per-trillion levels with no sample preparation. These characteristics mean that SIFT-MS can easily be applied to real-time detection of volatile organic air pollutants. This paper presents results from a air monitoring undertaken at Shu-Lin Elementary School in Taoyuan County, near Taipei, Taiwan R.O.C. from 19 to 21 July 2011.

SIFT-MS uses chemical ionization reactions coupled with mass spectrometric detection to rapidly quantify targeted VOCs. VOCs are identified and quantified in real time from whole-gas samples based on the known rate coefficients for reaction of the chemically ionizing species (so-called reagent ions) with the target analytes. The soft chemical ionization used in SIFT-MS yields a smaller range of product ions than is common in electron impact mass spectrometry (as used by gas chromatography mass spectrometry (GC-MS) , for example) . Hence the need for gas chromatographic separation of the sample is circumvented, speeding sample throughput and providing instantaneous quantitation of VOCs. Use of several rapidly switchable reagent ions to independently quantify target analytes also greatly reduces interferences, markedly increasing the specificity of SIFT-MS versus other whole-gas analysis technologies.

The high-speed analysis provided by SIFT-MS allows the instrument to provide continuous monitoring. Concentration data were extracted from full scan data for 48 compounds, many of which had been previously identified at the site using GC-MS. A number of these compounds exhibited interesting trends over the sampling period, including toluene, C_3-alkylbenzenes (e.g. mesitylene) , methanol, isopropyl alcohol, acetone and N,N-dimethylmethanamide. Measured concentrations range from sub-ppb to peak levels for methanol of nearly 250 ppb.

PTh-118 Development of UPLC-MS/MS method with large volume injection for simultaneous determination of regulated pesticides in drinking water.

13:30 – 14:40

Jun Yonekubo, Nobutake Sato

Nihon Waters K.K.,Japan

Keywords:
LC/MS/MS,pesticides,drinking water

Novel aspects:
Direct injection of large volume samples onto this system eliminated the need for sample preparation prior to analysis.This methods achieved good sensitivity,linearity,reproducibility for all targeted pesticides in regulation purpose.

Abstract:
Introduction

Rapid and highly sensitive analysis of drinking water is essential for protecting human health and well-being. The assurance of clean safe drinking water has become more critical given the potential of accidental or international contamination, which has increased in recent years.

Highly efficient water treatment processes allow for the effective removal of the majority pesticides that have entered water sources, however, drinking water regulations still require testing to ultra-trace concentrations.

This requirement has led to multiple approaches for enriching samples before instrumental analysis, with solid phase extraction (SPE) prior to LC/MS/MS a popular choice. In addition to this online pre-concentration and large volume injection, using specialized injection systems have been employed to introduce samples to LC/MS/MS systems. These techniques have been be very successful, but can add time, resources, and complexity to analysis.

Cleaner aqueous samples, such as drinking water are highly compatible for direct injection onto a LC/MS/MS system, but large multi-analyte determinations require extremely fast systems with high-sensitive detection.

In this study, 30 pesticides which was regulated as Complementary Items by Ministry of Health, Labour and Welfare (MHLW) in Japan, is targeted. The use of large volume direct injection for the rapid, precise, and high-sensitive analysis of these 30 pesticides in drinking water is developed.

Experimental

UPLC conditions
LC System : ACQUITY UPLC H-class, Run time : 10 min.
Column : ACQUITY UPLC HSS C18, 2.1 x 100 mm, 1.8 um
Mobile phase : 0.05 % HCOOH aq. / CH_3OH gradient
Flow rate : 0.4 mL/min. Injection volume : 50 uL
MS conditions
MS System : Xevo TQD
Ionization mode : positive/negative switching
Capillary voltage : 0.5 kV, Source temp. : 150 &#8451 ;
Desolvation gas/temp. : 1000 L/Hr (400&#8451 ;) , Acquisition mode : SRM

Results

Sample throughput
Rapid this system separations allowed a high-throughput analysis with all pesticides of interest eluting before 6.5 min and total runtime of 10 min for each sample.

This system was operated with load-ahead enabled. This allows for the next sample to be ready to inject immediately after the previous sample has completed, which helps optimize instrument efficiency.

High-sensitive detection
Detection of pesticides to lower concentrations was achieved using large volume injection into Xevo TQD. Up to 50 uL injection, there are not observed any deterioration of peak shape and separation. Amount of on-column pesticides with large volume (50 uL) injection was 10 times higher than normal (5 uL) injection. Improvement of sensitivity which was performed to increase of injection volume is able to a few or several ppt level detection.

This level of sensitivity allows detection of pesticides to 1/100 of a desired value and is match for requirement of the regulation.

Linearity and Reproducibility

External calibration (point) of target pesticides was performed at concentrations around the regulatory level for each pesticides. Good linearity was achieved for all compounds analyzed with typical coefficient of determinations (r^2) of > 0.99.

According for the procedure of testing pesticides in drinking water, Reproducibility of this method was verified at 4 or 20 ppt concentrations. MHLW testing procedure mentioned in the method accuracy, Coefficient of variation (CV) at 1/100 of each pesticides desired value, It should be 20% or less in principle. % RSD of all compounds were within permissible range.

Conclusion

Direct injection of large volume samples onto this system eliminated the need for sample preparation prior to analysis.
This methods achieved good sensitivity and linearity, reproducibility for all targeted pesticides in regulation purpose.

PTh-119
11:10 – 12:20

Development of Automated Identification and Quantification System with a Database

Kiwao Kadokami[1], Terumi Miyazaki[5], Chiaki Karaki[1], Daisuke Jinya[2], Tomomi Iwamura[2], Kaori Ohkubo[4], Takashi Miyawaki[3], Yoko Nakazono[4], Kouji Takahashi[3]

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Keywords:
GC-MS, LC-TOF-MS, TIM, Comprehensive analysis

Novel aspects:
Novel GC-MS and LC-TOF-MS databases that can identify and quantify micropollutants in environmental and food samples without the use of standards are reported.

Abstract:
To thoroughly examine environmental pollution by chemicals, a large number of chemicals should be analyzed. However, because it is known that thousands of chemicals exist in the environment, it is very difficult to analyze all of toxic chemicals simultaneously because of the huge cost and the large amount of labor needed using existing methods. In order to solve this challenge, we have developed two Automated Identification and Quantification System with a Database (AIQS-DB) for GC-MS and LC-TOF-MS, which can measure a large number of chemicals without the use of standards.

In the AIQS-DB for GC-MS, GC retention times, calibration curves, and mass spectra of nearly 1000 semi-volatile organic compounds were registered, and the GC retention times of registered chemicals in actual samples were predicted from the retention times of *n*-alkanes measured before sample analysis. Differences between predicted and actual retention times were less than 3 s, an accuracy that is nearly identical to that obtained by analysis of standard substances. The reproducibility of quantification was examined on four GC-MS systems in four laboratories using 114 substances with a wide range of physicochemical properties and which are usually difficult to measure with a GC.

When the performance of a GC-MS passed the designated criteria, the reproducibility of quantification results of 47 substances was almost the same as that obtained by a conventional method that uses calibration curves prepared before sample analysis. Although the reproducibility of 42 substances was slightly lower than that by the conventional method, the reproducibility is reliable enough except for in cases that require a high reliability. However, the dispersion of results of 25 substances was large under the GC conditions used. The causes that affect the dispersion of quantification results are functional groups and the structure of molecules. The effects of the functional groups are hydroxyl amino > nitro group. The effects of the number of them are multiple > single. The position of them can also have different effects : farther > nearer. In the case of the structure of molecules, the effects are as follows : side chain > straight chain > single benzene > multiple benzenes. In terms of sensitivity, more than 90% of the chemicals in the AIQS-DB could be detected at a sensitivity sufficient for all practical purposes (100 pg or less) .

The AIQS-DB for LC-TOF-MS has been constructed according to the same concept as the GC-MS method. In order to measure as a large number of chemicals as possible, the combination of an ODS column and acetate buffer-methanol eluent (pH 6.8) and positive ionization were used. Retention times of chemicals in the AIQS-DB were predicted from relative retention times of internal standards. Differences between predicted and actual retention times were within +/- 30s as long as LC conditions were constant. From correct prediction of retention times and high-resolution mass spectra obtained by TOF-MS, identification of chemicals can be consistently performed. Quantification values are also constant as long as LC conditions are constant ; reproducibility (RSD) was less than 20%, which is slightly worse than the conventional method that calibration curves are prepared just before sample analysis. However, the value of simultaneous measurement of a large number of chemicals compensates for lower accuracy.

Because each chemical in both AIQS-DB systems can be determined in 1 h, hydrophilic and hydrophobic micropollutants in samples can be measured efficiently and inexpensively by using both the AIQS-DB systems, and to which new substances can easily be added.

PTh-120 Metal speciations in environmental and clinical applications

13:30 – 14:40

Yeuk-Ki Tsoi, Kelvin Sze-Yin Leung

Hong Kong Baptist University, Hong Kong

Keywords:
elemental-speciations, LC-ICP-MS, IIP, SPME, DRC

Novel aspects:
high-throughput hyphenated instrumentation ; SPME-LC for speciation, modified material for sample preconcentration and simultaneous speciation ; selectivity modulation using ion-imprinting technique

Abstract:
In the vast subject of trace analysis, evolutionary instrumentation and material developments are the current trends to re-invent the definitions of sensitivity and selectivity in the field of elemental speciations, which in fact has become an indispensible tool for environmental management and clinical monitoring. In the world's raising knowledge and demands for the quality of human health, the strategies of method development is conforming to the stringent requirements of analytical standards. A diversity of research work in this presentation aims to facilitate convenient execution of speciation protocols in routine monitoring processes. The novelty is exhibited at four levels, featuring high-throughput instrumental methods, simplified extraction with commercial tools, modified material for specialized applications and modulation of selective material with ion-imprinting technique.
In the first of our presented work, seven species of these elements, namely Cr (III) , Cr (VI) , As (III) , As (V) , monomethylated As, Se (IV) and Se (VI) , were simultaneously determined by liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) with dynamic reaction cell (DRC) that offers optimized sensitivity at sub-ppb levels to the selected elements. The high through-put of this method has fulfilled the hustle routine of environmental water analysis [1] .
Aiming at clinical monitoring application, the second part demonstrates a method of solid-phase microextraction-liquid chromatography (SPME-LC) developed for MeHg and EtHg speciation in complex biological matrix [2] . Organomercury speciation in patients' urine has revealed source and pattern of Hg exposure which should allow rapid diagnostic whereby appropriate clinical treatment can be applied.
The third part presents a novel laboratory-made SPE column packed with tetrabutylammonium hydroxide-immobilized activated carbon (AC-TBAH) for Se (IV) and Se (VI) enrichment. The assigned anion-exchange functionality on the porous AC surface offered outstanding enrichment factors for ppt-level speciation with LC-ICP-DRC-MS [3] . Speciation analysis in natural water samples further validated the robustness of the material which hence represents a low-cost substitute for anion exchange resins for routine applications.
Material design has been proven very useful for modulating selectivity for trace element analysis via the synthesis of ion-imprinting polymer (IIP) , such as one demonstrated in final part of the presentation. After a series of selectivity investigations, imidazole-based arsenic imprinted polymer was selected for selective extraction and analysis of trace As ions in environmental application [4] . Tunable selectivity through monomer selection revealed a promising advantage of imprinting technique for future speciation method development. With growing maturity of speciation research, routine implementation in environmental and biological areas should make progress towards a sustainable human well-being.

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[2] Y.-K. Tsoi, S. Tam, K. S.-Y. Leung, J. Anal. Atom. Spectrom. 25 (2010) 1785.
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[4] Y.-K. Tsoi, Y.-M. Ho, K. S.-Y. Leung, Talanta 89 (2012) 162.

PTh-121
11:10 – 12:20

Occurrence of 92 pharmaceuticals in river water in agricultural or urban areas

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Public Works Research Institute, Tsukuba, Japan

Keywords:
Pharmaceuticals, Sewered Area, Unsewered Area

Novel aspects:
The objective of this study is to determine the occurrence of pharmaceuticals in river waters characterized by land use of their river basins, such as agricultural or urban areas.

Abstract:
In recent years, physiologically active substances (e.g., pharmaceuticals) detected in the water environment have become an emerging public concern. Human-use pharmaceuticals enter raw sewage via urine and feces or by improper disposal. These pharmaceuticals are discharged from private households and hospitals, and eventually reach wastewater treatment plants. If pharmaceuticals are only partially eliminated, residual quantities enter the water environment. However, little information is available about the occurrence of pharmaceuticals in river water. The objective of this study is to determine the occurrence of pharmaceuticals in river waters characterized by land use of their river basins, such as agricultural or urban areas.

We conducted sampling on six fine days at two stations of a river in an agricultural area (St.1 and St.2) and two stations at a river in an urban area (St.3 and St.4). The catchment areas of each sampling site are 4.6km^2 (St.1), 4.1km^2 (St.2), 3.9km^2 (St.3) and 5.4km^2 (St.4). About 90 % of land utilization of the catchment area of St.1 and St.2 consists of rice field, dry field, artificial forest, and broadleaf forest. Land utilization of the catchment area of St.3 and St.4 consists of a residential area (38% and 36%), school zone, open space, dry field, native grassland and artificial forest (54% and 56%), respectively. The catchment area of St.1 and St.2 is an unsewered area, and St.3 and St.4 are partly sewered areas. The percent of sewered population of the catchment area of St.3 and St.4 is 74% and 31%, respectively. The populations of each sampling site are 1,062 persons, 407 persons, 25,71 persons and 28,171 persons for St.1, St.2, St.3 and St.4, respectively. The population densities of the river basins are 231 persons/km^2 of St.1, 99 persons/km^2 of St.2, 6,594 persons/km^2 of St.3 and 4,661 persons/km^2 of St.4.

The analytical method of pharmaceuticals for river water samples is as follows : first, a 200ml sample was filtered through a 0.7-μm pore size glass fiber filter. After filtration of the sample, solid phase extraction was performed using an Oasis HLB cartridge. Subsequently, the cartridge was eluted with methanol. After solvent removal, the residue was dissolved in acetonitrile/water solution, which was then analyzed by LC/MS/MS. The procedure demonstrated in this study is innovative in terms of simultaneous analysis of 92 substances.

We identified a total of 48 substances (e.g., anti-inflammatory drugs, anti-epileptic drugs, antihypertensives, antibiotics, etc.) in river waters in agricultural and urban areas. The observed average concentration of pharmaceuticals ranged from 0.6ng/L to 14ng/L, 0.2ng/L to 52ng/L, 0.2ng/L to 670ng/L and 0.4ng/L to 1,500ng/L for St.1, St.2, St.3 and St.4, respectively. The numbers of pharmaceuticals detected in surveyed rivers were different. We found 15, 25, 37 and 45 for St.1, St.2, St.3 and St.4, respectively. In agricultural areas (St.1 and St.2), the numbers are much less than those in urban areas (St.3 and St.4). And also, the concentration of detected pharmaceuticals in agricultural areas (St.1 and St.2) is lower than urban areas (St.3 and St.4) and more pharmaceuticals were detected in St.4 than in St.3, which might be caused by the insufficient and discharge of wastewater in the catchment area of St.4. The number of detected pharmaceuticals is large when the population density is large, which is assumed to be caused by the pharmaceuticals used in society. In agricultural areas, detected numbers of pharmaceuticals were much less than those in urban areas and the concentration of detected pharmaceuticals in agricultural areas was also lower than in urban areas.

PTh-122

13:30 – 14:40

Application of tandem mass spectrometry for the structure confirmation of a wide range of peptides synthesized by cyanobacteria Woronichinia naegeliana

Beata Bober[1,2], Zbigniew Lechowski[2], Jan Bialczyk[2], Piotr Suder[3], Ken-ichi Harada[1]

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Keywords:
Cyanobacteria, peptides, MS/MS, structure confirmation

Novel aspects:
The structure confirmation of a wide range of bioactive peptides synthesized by cyanobacteria Woronichinia naegeliana can be successfully performed using MS/MS.

Abstract:

Introduction

The eutrophication of water caused by human activity has influenced the increasing of mass appearance of cyanobacteria. The cyanobacterial blooms are undesirable because of ecological as well as health reasons. Cyanobacteria have a remarkable ability to synthesize and release into the water a wide range of bioactive secondary metabolites. Most of them are oligopeptides, which in terms of molecular structure are divided into linear (aeruginosins, microginins) , cyclic (anabaenopeptins, cyanopeptolins, microcystins, cyclamides) and multicyclic (microviridins) . Some of them have been recognised to cause accute or chronic toxicity and might be reasons for serious health problems, others could be an attractive resources for new drug discoveries. The determination of these compounds is of great significance because the cyanobacteria that produce them often occur in reservoirs of water designated for consumption. *Woronichinia naegeliana* (Unger) Elenkin is a species of cyanobacteria belonging to *Chroococcales* and is appearing increasingly frequently in fresh water areas worldwide. Outbreaks, particularly in late summer and autumn, have been confirmed in Europe, North America and Australia. However, to date its secondary metabolites have not yet been determined adequately. As a result of the potentially significant consequences that can result from a bloom of *W. naegeliana,* research has been undertaken to identify its secondary metabolites. Taking into consideration limitations of applicated analytical methods such a high performance liquid chromatography (lack of standards) and nuclear magnetic resonance (a high amount of sample is required) , only the using tandem mass spectrometry (MS/MS) with electrospray ionization allows to confirm structures of compounds synthesized by *W. naegeliana* with a high degree of specifity.

Method

Field samples of *Woronichinia naegeliana* were collected from blooms in Dobczyce Reservoir (southern Poland) . The obtained extract from the lyophilized cyanobacteria cells was concentrated by solid phase extraction (SPE) . Separation and preliminary analysis of the samples were carried out using a high performance liquid chromatography system with photodiode array detector (HPLC-PDA) . The isolated fractions were analyzed with a mass spectrometer with an electrospray ion source (ESI-MS) . The positive-ion mode was applied. The scan range was m/z 200-1400 in the MS and MS/MS modes. Because purified standards of the majority of metabolites produced by cyanobacteria are not available for identification, confirmation of the identity of a given compound was done by interpretation of the MS/MS spectra.

Results and Discussion

The obtained product ions were quite effective for the amino acid sequencing of linear as well as cyclic cyanopeptides. In the case of cyclic peptides, the characteristic fragment or series of fragment indicating the cleavages at definite peptide bonds were helpful for the interpretation of fragmentation pattern. On this base the peptides isolated from field samples of *W. naegeliana* fell into four classes : microginins (microginin 757, microginin 91E, microginin FR3, microginin FR 4) , cyanopeptolins (cyanopeptolin B, cyanopeptolin C, cyanopeptolin D, cyanopeptolin 880, micropeptin 478-B, micropeptin 88D) , anabaenopeptins (oscillamide B) and microcystins (trace amounts of microcystin-LR) . The molecular masses of the determined metabolites range from 700 to 1100 Da. The particular groups of structurally related compounds showed similarity of the fragmentations patterns. These results confirm the usefulness of MS/MS for the determination of linear and cyclic cyanopeptides with a high structural diversity.

PTh-123
11:10 – 12:20

A search for active ingredients in cigarette smoke that modify significant biomolecules

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Keywords:
Cigarette smoke extract, L-tyrosine, LC/MS, LC/MS/MS, GC/MS

Novel aspects:
We found out that active ingredients in CSE readily react with Tyr to form various Tyr derivatives containing a new compound, suggesting that cigarette smoke can modify biogenic amino acids.

Abstract:
Cigarette smoke contains a number of toxic chemicals, many of which contribute to the pathogenesis of smoking-related diseases such as chronic obstructive pulmonary disease, vascular disease and cancer. Modification of biomolecules by the harmful chemicals present in cigarette smoke is thought to mediate the adverse health effects of smoking. The chemical analysis of such active ingredients in cigarette smoke is one of the most challenging tasks for analysts, and extensive work has been done using many analytical techniques. Despite such work, there have been no reports regarding the analysis of reaction products from the compounds incigarette smoke and functional biomolecules.

The purpose of this study is to search for biomolecules modified by active ingredients in cigarette smoke. We tried to identify the products formed by reaction of gas-phase cigarette smoke extract (CSE), from which nicotine and tar had been removed, with L-tyrosine (Tyr) on the assumption that Tyr is a key target amino acid in proteins for cigarette smoke toxicity. A highly sensitive LC/MS/MS system was utilized for detection and identification of trace amounts of there action products. We also tried to identify and quantify the active ingredients in CSE by GC/MS or LC/MS.

CSE was prepared by bubbling into phosphate-buffered saline (PBS) (1 mL per three cigarettes) the main stream of smoke (gas phase) from which the particulate phase including tars and nicotine had been removed 99.998% by passage through a Cambridge filter. The pump flow rate was kept constant (1 L/min) and smoke was bubbled only for 1 min after lighting a cigarette. The resulting solution was designated the 100 % CSE solution and stored at -80&#8451 ; until it was used. AQuattro Premier triple-quadrupole LC/MS (Micromass, Manchester, UK) with an ESI source was used for the positive and negative ion mode Q1 scan and MS/MS analysis coupled to the Alliance HT 2795 Separations Module (Waters Co., Milford, MA, U.S.A.) . Amass spectrometer (Automass SUN, JEOL Ltd., Tokyo, Japan) equipped with a GC (6890N, Agilent Technology Inc., Santa Clara, CA, U.S.A.) was used for identify and quantify the active ingredients in CSE.

We found that CSE readily reacts with Tyr at body temperature (37&#8451 ;) to form various Tyr derivatives, which can be detected using a LC/MS and LC/MS/MS. From among these derivatives, we could identify two acetylated compounds, *N*-acetyl-Tyr and *O*-acetyl-Tyr, and a new compound, *M*r251 (Tyr+70) .

Next, to identify and quantify the compound of *M*r 251 (Tyr+70) in CSE we used GC/MS analysis. We found that two peaks indicate *M*r 70 from *m/z* 70 chromatogram of CSE. From the result of the library search of their mass spectra, we identified the peak for t_R 9.5 min as crotonaldehyde and the peak for t_R 6.3 min as methyl vinyl ketone, as candidate ingredients. These retention times were the same as those of their respective authentic samples. Thus, to clarify the structure of compound Tyr+70, the authentic crotonaldehyde and methyl vinyl ketone were directly reacted with Tyr at 37&#8451 ; . The reaction products were analyzed by LC/MS in the SRM mode. The peak of compound Tyr+70 appeared on the SRM spectra of the mixed solution of Tyr and methyl vinyl ketone, but was hardly detected on that of the mixed solution of Tyr and crotonaldehyde. The analytical data of compound Tyr+70 synthesized by reaction of methyl vinyl ketone with Tyr completely agreed with those of compound Tyr+70 obtained by reaction of CSE with Tyr. This indicates that methyl vinyl ketone in CSE reacts with Tyr to produce compound Tyr+70. The structure of compound Tyr+70 was identified as *N*- (3-oxobutyl) -Tyr [3- (4'-hydroxyphenyl) -2- (3 "-oxobutylamino) propanoic acid] .

PTh-124
13:30 – 14:40

Development of smoke diagnostic assays: When the smoke clears, will it end up in the wine bottle?

Yoji Hayasaka, Gayle Baldock, Mango Parker, Kevin Pardon, Cory Black, Markus Herderich

The Australian Wine Research Institute

Keywords:
volatile phenols, phenolic glycosides, HPLC-MS/MS, smoke, bushfires

Novel aspects:
The newly developed methods based on the analysis of grapes and wine for phenolic glycosides induced by smoke exposure were proven to be sensitive and reliable as smoke diagnostic assays

Abstract:
Smoke from forest/bushfires across winemaking regions in Australia as well as overseas has caused much concern among grape-growers and winemakers. Wines made from smoke-affected grapes have been described as 'smoky', 'dirty', 'ashtray' and 'burnt'. To aid purchasing, harvesting and winemaking decisions following a smoke event, winemakers and grape-growers clearly need reliable diagnostic strategies to assess the impact of smoke exposure in grapes as early as possible, ideally before harvest.

Measurement of the smoke marker compounds guaiacol and methylguaiacol in grapes has been commonly carried out as the smoke diagnostic assay and can identify severely smoke-affected samples. However winemakers have reported increasing numbers of wine samples developing undesirable smoke-related characters during and after winemaking, even when grape guaiacol concentration was as low as natural abundance. Additional challenges are that guaiacol can be found in non-smoked grapes as a natural component, and is extracted from toasted oak, so interpretation of wine data is complicated. These observations strongly suggested that the better smoke marker compounds to assess the extent of smoke exposure on grapes and wine were needed.

To find new smoke marker compounds and develop practical and reliable smoke diagnostic assays, various mass spectrometric techniques were applied to the following experiments ;
· analysis of smoke generated by a forest fire to find major smoke components
· uptake of the major smoke components by grapes
· metabolism of the major smoke components in grapes
· identification of metabolites of the major smoke components in grapes
· extraction of the metabolites from grapes to wine
· evaluation of the metabolites as smoke marker compounds
· development of methods for the quantification of the metabolites used as smoke diagnostic assays

As a result, various glycosidic forms of guaiacol, methylguaiacol, syringol, methylsyringol, *o*-, *p*- and *m*-cresol, and phenol were selected as smoke marker compounds. Advantages of the use of these glycosides were as follows ;
· when a grapevine is exposed to smoke, the amount of the volatile phenols taken up by grapes can be related to the intensity and duration of smoke exposure
· once taken up by grapes, the volatile phenols are rapidly metabolized into their more stable and non-volatile glycosidic forms
· the smoke induced glycosides persist and accumulate in grapes until the time of harvest, therefore, the amount of the grape glycosides can be correlated to the intensity of smoke exposure, and possibly also used to quantify extended or repeat smoke exposure
· the phenolic glycosides are not present in non-smoked grapes and are also not found (unlike free guaiacol) in oak in significant concentrations ;
· the phenolic glycosides are easily extracted into wine and act as a pool of precursors to release volatile phenols during alcoholic and/or MLF fermentation, aging, and storage. This implies that measurement of phenolic glycosides can be used to estimate the potential of grapes to produce smoke-related taints after fermentation and at the time of consumption.

PTh-125
11:10 – 12:20

Ions Observed in DART-MS Analysis of Pharmaceuticals Containing Various Functional Groups on Normal and Reverse Phase TLC Plates

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Keywords:
TLC-MS, DART, Pharmaceuticals, PPCPs

Novel aspects:
This report is the first detailed study on DRAT mass spectrometry of pharmaceuticals containing various functional groups in TLC-DART/MS.

Abstract:
Thin Layer Chromatography (TLC) is one of the most popular chromatographic methods and widely used as separation and purification techniques in a variety of fields such as pharmaceutical science, forensic medicine, organic synthetic chemistry, botanical science and environmental analysis. TLC is an inexpensive and easy operation method in comparison to high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). However, the identification of compound by TLC analysis often becomes difficult because of its low specificity. To dissolve this problem, mass spectrometry (MS) has been generally used for identification of compounds separated on a TLC plate. In the previous time, the structural elucidation by MS analysis has been carried out after extraction of the target compound from a TLC plate. This off-line TLC-MS method is the relatively much time-consuming technique. Thus, various ionization modes have been investigated to analyze the compounds on TLC plate directly, so far the following ionization techniques have been utilized in a direct sampling TLC-MS analysis ; Fast atom bombardment ionization method (FAB), Matrix associated laser desorption ionization method (MALDI), Electrospray ionization method (ESI), Desorption electrospray ionization method (DESI), Direct analysis in real time ionization method (DART), and so on.[1)]

Among the direct sampling TLC-MS analyses, TLC-DART/MS seems a relatively easy method if a target compound is not non-volatile, because the ionization of compound on a TLC plate can be performed under atmospheric pressure without solvents and matrixes. However, it is relatively difficult and inconvenient to cut a glass type of TLC plate to a narrow width strip (below ca.10mm) after separation of samples on a TLC plate. A new type of DART ion source was introduced in 2009, which allowed the angle of DART gas stream to be adjusted. The new model shall make it easy to access to more wide surfaces compared with the conventional DART ion source (horizontal type).[2)]

On the other hand, the only protonated molecules ions have been discussed in the majority of publications on DART mass spectrometry. In the case of TLC-DART/MS, there are few publications about the detailed investigation on DART mass spectra of compounds containing different functional group.[3)] Therefore, we started investigation about the characteristics of the DART mass spectrometry to use the TLC-DART/MS method for analysis of pharmaceuticals and personal care products (PPCPs).

At the first time, we selected the typical pharmaceuticals containing various functional groups, which occurred as the major PPCPs in aquatic environments. Most of compounds were obtained by extraction with methanol from the ethical pharmaceutical products, if necessary, were purified by re-dissolving with acetonitrile and/or solid-phase extraction. The silicagel60 F_{254} and RP-2 F_{254} were used as normal-phase and reverse-phase TLC plates, respectively. Both of them were a glass type of TLC plates (20 X 20 cm, stationary-thickness : 0.25 mm) purchased from Merck. The glass strips (100 mm X ca.10 mm) were prepared by cutting TLC plates and washed by development of methanol in a glass developing chamber. After the sample solutions were prepared in a concentration level of 0.1 to 5 mg/mL using appropriate solvent, a portion of 1 or 2 μL was applied on a TLC plate using a glass capillary. DART-MS system consisted of DART-SVP-100 (IonSence) for ionization of samples and Accu-TOF mass spectrometer (JMS-T100LC, JEOL) for acquisition of mass spectral data.

In this conference, we will present the information on DART mass spectrometry of pharmaceuticals spotted on normal- and reverse-phase TLC plates.

Reference
1) Sy-Chyi Cheng, et.al., J. chromatogr. A, 1218, 2700-2711 (2011)
2) E.S. Chernestsova, et.al., Rapid Commun. Mass spectrum., 25, 2275-2282 (2011)
3) E.S. Chernestsova, et.al., J. Anal. Chem., 66 (13), 1348-1351 (2011)

PTh-126
13:30 – 14:40

Concentration profiles of PCB congeners in Steller sea lion, Hokkaido, Japan

Keiko Kubo[1], Katsuyuki Yamaguchi[2], Wakana Yamada[3], Tsuyoshi Ishinazaka[4], Masaki Mitsuhashi[6], Kaoru Hattori[5], Shunitz Tanaka[1]

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Keywords:
PCBs, congeners, fetus, liver, blubber

Novel aspects:
The distributions of PCB congeners in SSL were described. The concentration profiles of PCB congeners in the liver and blubber were different among males, females and fetuses.

Abstract:

Introduction

PCBs still have been the major contaminants in the marine mammals at the top of the marine food-web. Especially, liver and blubber were thought to be most accumulative organs of PCBs in their body. The high risks of PCBs on the immunity obstruction and the cacochymia in marine mammals are feared. Therefore, more detail survey about PCB congeners in marine mammals is necessary.

In this study, PCB congeners in the liver and blubber of Steller sea lion (*Eumetopias jubatus* : SSL) from the coastal Hokkaido, Japan were measured by HRGC-HRMS to describe the distribution of PCB congeners in the males, females and fetus of SSL.

Materials and Method

The liver and bluber samples of SSL were obtained from commercial fishery-related control killed and bycatch animals at the Shakotan area and Nemuro Strait, Hokkaido, Japan, during winter season in 2008 and 2010. These samples were measured by an isotope dilution method with ^{13}C-labeled internal standard substances. The liver samples of eight males, two females and three fetuses were analyzed. The blubber samples of four males and three females and three fetuses were analyzed. These samples include three pairs of mother and fetus.

Results and Discussion

In the males, #153 (in IUPAC#) was predominated in the both organs of the liver and blubber, followed by #138, #99 and #118. The concentration levels of #28, #31, #177 and #199 were appeared to be considerable differences between these tow organs. It seemed that each organ had specific accumulative property of PCB congeners. The total PCB concentrations (ΣPCBs) of males in the liver and blubber were 3,100±1,300 ng/g-fat and 3,000±830 ng/g-fat, respectively.

Between genders, the concentration profiles of major congeners in the blubber were differed, significantly. Meanwhile in the liver, there were no obvious differences in the congener profile between the male and female. In general, gender differences depend on the experience of pregnancy and lactation. ΣPCBs of the female in the liver and blubber were 1,200±810 ng/g-fat and 910±410 ng/g-fat, respectively. ΣPCBs in female were 61%-71% less than in males.

We analyzed PCBs in the liver and blubber from three pairs of mother and fetus to describe the detail profiles and to compare the concentrations between them. In the blubber, #153 and #138 were dominated congeners except one fetus. The major congeners in the blubber and liver of fetus were #153, #138 and #118 except one fetus. The average of ΣPCBs in the liver and blubber of the fetus was 710±430 ng/g-fat and 1,600±900 ng/g-fat, respectively. It was explained that ΣPCBs in the blubber of fetus was about 27%-85% higher than those of mother. These fetuses already have been contaminated during gestational period. It was thought that the concentration levels of these fetuses might depend on each mother's PCB levels.

The concentration profiles of congeners in the liver and blubber were different between the liver and blubber of males, the blubber of genders, each pair of mother and fetus. The level of ΣPCBs in male SSL from Hokkaido was as the same level as the report in 14 years ago (Kim et al.,1996) . The PCB levels of congeners in SSL from Hokkaido showed how PCBs still remained in marine environment of the coastal Hokkaido. More investigations of PCB congeners in SSL are needed to study the mechanism of the accumulation and metabolism.

PTh-127
11:10 – 12:20

Investigation of metabolites formed during activated sludge treatment of clarithromycin

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Keywords:
clarithromycin, wastewater, activated sludge treatment, metabolite

Novel aspects:
During activated sludge treatment, sulfate conjugation that is one of the phase II reactions observed as mammal's drug metabolism may occur in CAM.

Abstract:
The occurrence of antibiotics in aquatic environments is of ecotoxicological concern because of potential ecosystem alteration. Prolonged exposure to low doses of antibiotics leads to the selective proliferation of resistant bacteria, which could transfer the resistance genes to other bacteria species. Since most antibiotics used in human treatment are eventually disposed of with wastewater, wastewater treatment plants play a key role in removing antibiotics and preventing them from reaching the receiving water bodies.

Clarithromycin (CAM) is a half synthetic macrolide antibiotic which is induced from the antibiotic erythromycin. In Japan, CAM has been detected at low microgram / liter in some untreated wastewater samples. It is understood that CAM is one of the drugs that has ecological risk in aquatic environments. In most of the investigations reported to date, the efficiency of CAM removal during wastewater treatment is determined by measuring the disappearance of the parent compound. But little attention has been given to the identification and quantification of transformation products formed during wastewater treatment.

In the present study, the activated sludge treatment that was the typical wastewater treatment method was reproduced in the laboratory, and screening of major metabolites of CAM was conducted. To investigate the microbial degradation of CAM in the aeration tank, a batch reactor (5 L) loaded with freshly collected activated sludge from the municipal wastewater treatment plant was spiked with the parent compound at a concentration of 20 μg/L. Negative control experiments (without CAM, with activated sludge) and abiotic experiments (with CAM, without activated sludge) were also performed. For the screening of metabolites of CAM using the liquid chromatography / tandem mass spectrometry (LC/MS/MS) system equipped with an electrospray ionization (ESI) source, the samples were pre-concentrated by solid-phase extraction.

Analysis of samples from batch cultures with activated sludge revealed the formation of a metabolite with *m/z* 828 (retention time of 15.70 minutes) using LC / (+) ESI-MS [corresponding to protonated molecule $(M+H)^+$] . This metabolite was not observed in negative control and abiotic samples, suggesting that this peak is unique to the biotransformation of CAM in the presence of activated sludge. The protonated molecule of this metabolite (*m/z* 828) is hereinafter termed as M828. Because CAM was detected at *m/z* 748 (retention time of 15.35 minutes, hereinafter termed as P748) as the protonated molecule $(M+H)^+$, M828 increased 80 units more than P748. To facilitate identification of M828, fragmentation studies on P748 and M828 were performed by LC / (+) ESI-MS/MS. A minimum fragment ion to which 80 units shifted in the fragment ion of M828 compared with the fragment ion of P748 was *m/z* 238. This fragment ion corresponds to fragment ion *m/z* 158 of P748. There is a possibility of causing the shift of 80 units in M828 with desosamine because fragment ion *m/z* 158 of P748 is derived from desosamine, which is one of the sugars that composes CAM. Results from the analysis of LC / (-) ESI-MS reveal that *m/z* 826 was detected only with the sample from the batch culture with activated sludge [corresponding to deprotonated molecule $(M-H)^-$] . The deprotonated molecule of CAM was not detected. Hence *m/z* 826 in a negative ionization is presumed to be a deprotonated molecule of a metabolite of CAM, which corresponds to M828 in a positive ionization. A negative ionization is often caused by the elimination of protons in the acid group. One possible biotransformation that causes the change of +80 units is sulfate conjugation. During activated sludge treatment, sulfate conjugation that is one of the phase II reactions observed as mammal's drug metabolism may occur in CAM.

PTh-128
13:30 – 14:40

A NEW CHEMOMETRIC APPROACH FOR THE QUANTITATION OF STEROIDAL COMPOUNDS IN WASTEWATER BY GCxGC-TOFMS

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University of Helsinki, Finland

Keywords:
GCxGC-TOFMS, chemometrics, emerging organic contaminants, steroids, wastewater

Novel aspects:
The quantitation of unknown emerging organic contaminants with a chemometric model based on partial least squares regression equations, which were built up using six commercial steroid standards.

Abstract:
An increasing variety of chemical compounds including pharmaceuticals, personal beauty products and insect repellents are offered daily to consumers. The final destination for most of these compounds is the environment as raw compounds and their metabolites, some of which are potentially hazardous for the environment. In order to speed up the process of regulation and assessment of environmental threats, it is essential to develop rapid and efficient methods for the analysis of these contaminants.

In this study, exhaustive sample preparation method was first developed for the determination of steroidal species in the wastewater samples aiming at the clarification of the distribution of free and conjugated steroids in wastewater and suspended particles. Special emphasis was put on the applicability of comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS) to screen steroidal contaminants in wastewater. Although the resolution and sensitivity of GCxGC-TOFMS is superior over those of conventional GC-MS techniques, the lack of available commercial standards can hinder the usefulness of the technique, especially if the goal of the analysis is the quantitation of the identified compounds. Several approaches based on the use of surrogates can be found in the literature for the quantitation of compounds identified by MS, but frequently they lack the accuracy. In this study a new approach based on partial-least-squares regression (PLSR) was developed for the quantitation of emerging steroidal compounds in water and sludge samples taken from the largest wastewater treatment plant of Finland.

Six commercial steroidal compounds injected to the GCxGC-TOFMS at seven concentration levels ranging from 0.1 to 10 ng, were used as reference compounds for the development of the PLSR equation, which was successfully exploited for the determination of more than 40 steroidal compounds present in the analyzed samples.

PTh-129
11:10 – 12:20

High-Resolution Tandem Mass Spectrometry Analysis of the Interactions of Oligonucleotides with Selected River Basin Specific Pollutants

Janna Anichina, Andre Schreiber, Ron Bonner, Takeo Sakuma

AB SCIEX, Concord, Canada

Keywords:
Oligonucleotides, organic micro-contaminats, high resolution tandem mass spectrometry, micro-flow liquid chromatography, non-covalent interactions

Novel aspects:
For the first time the reactivity of selected organic micro-contaminants towards oligonucleotides was assessed using high resolution electrospray ionization tandem mass spectrometry combined with the micro-flow liquid chromatography.

Abstract:
Since a great number of organic compounds are annually released into the environment, the necessity to assess in a timely manner the potential risks associated with these chemicals along with the products of their environmental transformation is of high priority. In order to reduce the number of candidates for full-scale animal studies, potential toxicity of the compounds of interest can be rapidly assessed in simplified model systems. In this study we employed high resolution electrospray ionization tandem mass spectrometry (ESI-TOF/MS/MS) along with micro LC-ESI-TOF/MS to study interactions of selected river basin pollutants with model nucleic acids. The micro-contaminants were chosen in the context of the European Union Water Framework Directive (EU WFD) based on the assessment study for 500 organic substances observed in the four European river basins of the Elbe, Scheldt, Danube and Llobregat.

Two AB SCIEX LC-MS systems were used in this study: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument with Turbo V™ sources and Electrospray Ionization (ESI) probe in the negative ion mode. A high pressure micro-flow LC system was utilized for chromatographic separation. Interactions of two decameric oligonucleotides (ODNs), d(5'-GCGCATGCGC-3') and d(5'-GCGCGCGCGC-3') with diazinon, diuron, alachlor and bis(2-ethylhexyl) phthalate were investigated in the direct infusion mode and with the micro-flow LC separation. The AB SCIEX PeakView™ software with a prototype oligonucleotide fragmentation interpretation tool was used for the data analysis.

In this study we examined the interactions of two self-complementary decameric ODNs with four river basin specific pollutants. ESI-MS/MS analysis of the incubation mixtures of diuron with two selected ODNs indicated the formation of 1:1 adducts of both single-stranded oligonucleotides at a molar ratio of 10 or higher. Collected high resolution MS and MS/MS data were used to confirm the elemental composition of the ions of interest as well as to gain an insight into the structure of the adducts.

Tandem mass spectrometric measurements of the 1:1 adducts of diuron with both single-stranded ODNs demonstrated that their dissociation proceeds via the loss of a neutral diuron molecule at a relatively low value of the laboratory frame collision energy. When highly negatively charged diuron:ODN adducts (charge states of 5-, 6- or 7-) were exposed to the collision-induced dissociation (CID) we have also observed a competing charge-separation channel to produce a deprotonated diuron moiety and an oligonucleotide ion in (n-1)- charge state with n=5,6,7 respectively. Based on this observation we hypothesize a non-covalent mode of binding in the 1:1 complexes. Under the studied experimental conditions we did not detect diuron adducts with the ODN duplexes.

The results of the experiments performed with the studied ODNs and diazinon were similar to those observed for diuron-containing species. However, the formation of the 1:1 adducts was observed at a higher excess of diazinon (50-fold or higher). CID measurements of the diazinon adducts demonstrated an earlier (compared to diuron) CID onset which indicates a weaker binding with the ODNs. No detectable adducts of alachlor with either ODNs were formed even at 1000-fold excess of the compound.

This study has demonstrated an elegant application of high resolution tandem mass spectrometry combined with the micro-flow liquid chromatography for rapid screening of the reactivity of high priority environmental contaminants toward DNA models.

PTh-130 Formation of Hydroxy Polychlorinated Biphenyl

13:30 – 14:40

Shiho Fukuzawa[1], Takeshi Morita[1], Masahiro Shimase[1], Akinobu Kunitake[1], Takanori Sakiyama[2], Tameo Okumura[3]

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Keywords:
Hydroxy-PCB, PCB, formation mechanism, UV-light, soil

Novel aspects:
Congener specific formation of OH-PCB with UV-light in silica gel or without light in soil

Abstract:
Hydroxy polychlorinated biphenyl (OH-PCB) is one of an intravital metabolite of polychlorinated biphenyl (PCB) . Many cases about measurement OH-PCB in biological samples were reported and they pointed out that some of the isomer has an affect on thyroid hormone action or female hormone action. In the case of environmental samples Sakiyama et al.[1)] indicated the presence of OH-PCB in aqueous environment and bottom sediments. The result suggests that PCB is also hydroxylated in environment by physicochemical reaction or metabolic reaction of living organisms.

At our previous research[2)] on the actual situation of OH-PCB in the soil there are correlations between the OH-PCB concentrations and the PCB concentrations regardless of whether the soil is contaminated. We also reported that OH-PCBs are formed from mixture of PCBs (KCmix) under various conditions. No report is available that actually researched about the formation mechanism of OH-PCBs from dominant PCB congeners. In order to confirm the mechanism of OH-PCB formation, we selected 10 dominant congeners of PCB (#3, #12, #26, #31, #52, #101, #118, #153, #180, #209) as parent to hydroxylate under two conditions as follows. The first was irradiating UV-light to the silica gels, and the second was keeping the soils under light-shielding with addition individual PCB congeners. The OH-PCBs formed in the test samples were pretreatment through several steps and then methylated for analysis. The methoxy-PCBs were detected based on mass spectra characteristics after gas chromatography separation. In addition, they were identified by comparing their retention characteristics with methoxy-PCBs those were methylated from the available OH-PCB standards provided by reagent manufacturer (47 congeners) and Okumura synthesized (80 congeners) . We present the new findings about congener specific formation of OH-PCB that will be the help for the following studies on OH-PCB in environmental samples.

Reference literature
1) 16th Symposium on Environmental Chemistry, Sakiyama et al., 408-409 (2007)
2) 17th Symposium on Environmental Chemistry, Shimase et al., 480-481 (2008)

PTh-131
11:10 – 12:20

High Resolution LC-MS for Screening and Quantitative Analysis of Antibiotics in Drinking Water using an Orbitrap and Online Sample Preparation

Jonathan R Beck, Dipankar Ghosh, Charles T Yang

Thermo Fisher Scientific, San Jose, USA

Keywords:
Antibiotics, Orbitrap, Online SPE, PPCPs

Novel aspects:
High resolution mass spectrometry plus online pre-concentration to screen, quantify and confirm 27 antibiotics in water samples at low ppb levels

Abstract:
Most current methodologies for the quantitation of antibiotics in drinking water revolve around analysis using triple quadrupole platforms with offline sample preparation. The method described here utilizes LC-MS (/MS) with a Orbitrap instrument using high resolution accurate mass and online sample preparation. This work will describe a method to do screening and quantitation of 27 antibiotics at ppb and sub ppb levels in drinking water using online pre-concentration together with high resolution accurate mass confirmations of the compounds.

A standard curve containing 27 compounds was spiked in neat solution ranging from 100 pg/mL levels to 250 ng/mL levels was injected in triplicate and screening of different water samples (municipal tap water and bottled water) was analyzed for a targeted list of the 27 compounds. The sample was also screened for other possible unknown pesticides compounds. The spectrometer was set to a resolving power of 70,000 (FWHM) at m/z 200 in full MS mode to minimize matrix interferences, and data dependant all-ion-fragmentation (AIF) was collected to quantify and qualify. The data was then compared to a current MS/MS library for confirmation, and calibration curves were generated for the individual target compounds.

Calibration lines were generated for the compounds analyzed, and the limits of detection (LOD) varied from 100pg/mL to 500pg/ml based on the individual compounds based on a 1 mL injection. For confirmation of each compound, the exact mass of the compound, its isotope pattern and as well as the MS/MS spectrum produced were collected and compared against a fragmentation library which prevented "false positives " in the results. One of the main challenges using a high resolution accurate mass system is data mining. In this work we will show the data to screen, quantify and confirm in a single run therefore, spending less time on the instrument for repeat runs. Furthermore, the online pre-concentration step allows for low detection limits of these compounds without the time consuming steps of offline solid phase extraction of 1 L sample volumes.

PTh-132
13:30 – 14:40

Rapid screening and confirmation of emerging contaminants in UK river waters by UHPLC-IT-TOF

David R Baker[1], John Bagnall[2], Alan J Barnes[1], Barbara Kasprzyk-Hordern[2], Neil J Loftus[1]

[1]Shimadzu Co., Manchester, UK, [2]University of Bath, Bath, UK

Keywords:
river water screening environment I.T-TOF

Novel aspects:
Presented is a fast screening and confirmation method, applied to UK river waters, using UHPLC-IT-TOF with software (MetID) to aid in rapid and relatively straightforward interpretation of large data files

Abstract:
The extensive environmental distribution of emerging contaminants and their potential ecotoxicological effects at very low concentrations has attracted increasing interest amongst researchers, regulatory authorities and the public worldwide. Emerging organic contaminants constitute a diverse set of compounds including pharmaceuticals and personal-care products (PPCPs) , drugs of abuse and their metabolites, polar pesticides and their degradation/transformation products, perfluorinated compounds (PFCs) and organophosphorous flame retardants. The attention given to these contaminants is warranted as, amongst a number of reasons, many of these contaminants are relatively small molecules which may not be effectively removed during drinking water treatment.

Typically multi-residue methods by tandem mass spectrometry provide routine analysis in identifying emerging contaminants due to their sensitivity and selectivity. These are however limited to unit resolution and have low sensitivity in full scan. For these reasons the employment of high resolution mass spectrometry has become increasing popular in environmental analysis using full scan. Time of flight technology offers the possibly of accurate mass and isotope distribution analysis of targets compounds, however without specialist software, typically data analysis can be labour intensive.

In this study we address the discussed challenges by presenting a fast screening and confirmation method using UHPLC-IT-TOF (Shimadzu Corporation) with software (MetID, Shimadzu Corporation) to aid in rapid and relatively straightforward interpretation of large data files.

River water was collected from a major river in the UK and screened for the presence of over 100 emerging organic contaminants. Samples were extracted using Oasis HLB cartridges. UHPLC separation was achieved using a Kinetex XB-C18 100 x 2.1mm column, with 0.2 % formic acid in both the aqueous and organic (methanol) phases, and separated by binary gradient over 25 minutes. LCMS-IT-TOF data was acquired in the mass range 70-900 Da in both positive and negative ionisation modes using fast (100ms) polarity switching.

Preliminary data indicated several organophosphorous flame retardants were detected in addition to pharmaceutical compounds and an insect repellent compound. With these preliminary identifications, comparison to authentic standards will enable final confirmation without the expensive task of purchasing authentic standards for all possible screened compounds.

PTh-133 Degradation of Pentachlorobenzene by Fungi Screened from Nature

11:10 – 12:20

Ajeng A Sari[1], Sanro Tachibana[2], Kazutaka Itoh[2]

[1]The United Graduate School of Agriculture Sciences, Ehime University, Matsuyama, Japan, [2]Department of Applied Bioscience, Faculty of Agriculture, Ehime University

Keywords:
degradation, pentachlorobenzene, white rot fungi, metabolite

Novel aspects:
Limited information about degradation of pentachlorobenzene, a newly POPs, by white rot fungi and its metabolite products

Abstract:
Introduction: Pentachlorobenzene is used as an intermediate in particularly the fungicide pentachloronitrobenzene. It has also been used as fire retardant. This compound was added in 2009 to the list of POPs compounds covered by the Stockholm Convention. Considering pentachlorobenzene potential negative effects, it is necessary to address the environmental persistence of this pesticide and to develop effective methods for remediation by miroorganisms. White rot fungi are well known for their outstanding ability to produce extracellular oxidative enzymes e.g. lignin peroxidase (LiP) , manganese peroxidase (MnP) and laccase, which are involved in the degradation of either lignin than a wide range of pollutants include pentachlorobenzene. Other non-ligninolytic enzymes may participate in the transformation of pentachlorobenzene. The objective of these studies is to screen the fungi from nature having ability to degrade pentachlorobenzene on solid medium and in liquid medium, to investigate the enzyme activities secreted by selected fungi and to investigate the major metabolic products.

Experiment: Fungi screened from nature were inoculated for 7 d on a malt extract agar medium containing 1 mg pentachlorobenzene. Liquid culture experiment was conducted using 20 ml of malt extract medium in a 100 ml Erlenmeyer flask. After inoculation fungus for 7 d, 0.1 mM pentachlorobenzene solution was added and then incubated for 15 and 30 d. After a period of culture, the sample was extracted using ethyl acetate and purified using silica gel column chromatography eluent with hexane : dichloromethane (3 : 1) . The concentrate dissolved in toluene was analyzed by GC-MS Shimadzu QP-2010 equipped with a TC-1 column (30 m, id : 0.25 mm) . The carrier gas was helium delivered at a constant flow rate of 1.5 ml/min with column pressure of 100 kPa and interface temperature of 120 °C. The temperature program was started at 120 °C for 1 min, raised 20 °C/min to 180 °C, then 2 °C/min to 210 °C, then 5 °C/min to 310 °C, and maintained at 310°C for 3 min to allow the eluting peak to exit the column. For enzyme activity analysis, after period culture, the extracellular fluid was collected, filtered through a 0.2 µm membrane filter and measured by using Spectrophotometer. Enzymes that checked were laccase, MnP, LiP, 1,2 dioxygenase and 2,3 dioxygenase. For investigation of metabolic products, the samples were extracted with ethyl acetate and then the identification of metabolic products was performed in comparison with authentic standards.

Results and discussion: Cultivation of 4 fungi screened from nature on solid medium containing pentachlorobenzene indicated that growth of the fungi was obtained maximum of 100 % growth by 10th to 15th d. On solid medium, U80 showed the most-degrading pentachlorobenzene fungus. In liquid medium, U80 degraded pentachlorobenzene at 63% on 30 d. All enzymes tested were produced by U80 during pentachlorobenzene degradation which the high enzyme activity were obtained in dioxygenase and LiP. By comparing retention times and mass spectra with standard compounds by GC/MS, U80 produced 5 metabolite products. Pentachlorobenzene was initially dechlorinated to form tetrachlorobenzene, which was converted to trichlorobenzene, dichlorobenzene and chlorobenzene. Dechlorination was occured by presence of LiP. Moreover, dichlorobenzene could form 4-chlorophenol or 2,4-dichlorophenol. Dichlorophenol could be identified as a metabolite from dichlorobenzene or a product of dichlorobenzene dihydrodiol decomposition. It was caused by monooxygenase or dioxygenase because the initial attack on dichlorobenzene at the 1 or 2 position could form 4-chlorophenol or 2,4-dichlorophenol.

PTh-134 Analysis of thermal products of chlorpyrifos using LC/FTMS

13:30 – 14:40

Yoshinari Yamoto[1], Takanori Sakiyama[2], Hideya Kawasaki[1], Ryuichi Arakawa[1], Takeshi Nakano[3]

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Keywords:
chlorpyrifos, thermal products, LC/FTMS, isomers containing chrlorine

Novel aspects:
The thermal products of chlorpyrifos were identified to be its polymeric species containing a wide variety of structural isomers with different binding sites of chlorine atoms.

Abstract:
Introduction
Chlorpyrifos (O,O-diethylO-3,5,6-trichloropyridin-2-yl phosphorothioate) is an organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. Chlorpyrifos have been used in the lawn on a golf course. It was used for termite control as chlordane alternatives. Sakiyama *et al.* have reported that 2,3,7,8-TCDD- N-analogue is formed in pyrolysis experiments of chlorpyrifos by GC/HRMS analysis.[1] We should consider carefully burning scrap woods of houses contaminated by chlorpyrifos. It is suggested that the influence appears. In this study, the further thermal products are examined by LC/FTMS and the thermal degradation pathways are proposed.

Methods
All pyrolysis experiments were carried out in sealed brown glass ampoules (10 ml) with about 2 mg of chlorpyrifos and 3,5,6-trichloro-pyridinol (3,5,6-TCP) at temperature between 300 °C and 380 °C. After cooling to room temperature, the ampoules were opened carefully and the reaction products were extracted with toluene. The toluene was concentrated to 1 ml under gentle nitrogen stream. The thermal products of chlorpyrifos and 3,5,6-TCP are analyzed with an Exactive orbitrap mass spectrometer (ThermoFisher Scientific Inc.) equipped with positive electrospray ionization (ESI) probe and Accela LC system (ThermoFisher Scientific Inc.) with a column of Inertsil ODS-3 5 um 2.1×250mm (GL Science) . The best LC separation for the thermal products was achieved using (a) ammonium acetate aqueous solution (5 mM) and (b) acetonitrile for the gradient conditions.

Result and discussion
Analysis of chlorpyrifos after thermal treatment at 300 ~ 380 °C was performed by LC/FTMS. The peak intensity of 3,5,6-TCP (m/z= 195.91) was found to increase with decrease in that of chlorpyrifos. The results indicated that the formation of 3,5,6-TCP was probably due to the heating of chlorpyrifos. In addition, the thermal treatment of 3,5,6-TCP showed that the intensity of dimer, trimer and tetramer ions increased as the peak intensity of 3,5,6-TCP decreased in the mass spectra. The analysis of separation behavior for the LC-MS chromatogram indicated that these polymeric species contained some structural isomers. The new finding obtained from the present LC/FTMS measurements is that the heating of chlorpyrifos generates its oligomers by polymerization. The oligomers are shown to have a wide variety of isomers with different binding sites of chlorine atoms.

1) Sakiyama T, Weber R, Behnisch P, Nakano T, PreliminaryAssessment of Dioxin-Like Compounds In/From Chlorpyrifos - A PotentialPrecursor of the Pyridine Analogue of 2,3,7,8-TCDD, Organohalogen compounds : Vol. 73, 146-149 **(2011)**

PTh-135
11:10 – 12:20

Behavior of Hexabromocyclododecane (HBCD) stereoisomers in water,sediment, and biological samples.

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Keywords:
brominated flame retardants, 1,2,5,6,9,10-hexabromocyclododecane, LC/MS/MS, environment

Novel aspects:
HBCDs regional contaminations in Nagoya city were revealed by measuring river waters, sea waters, sediments, fishes and breast milks.

Abstract:
1,2,5,6,9,10-hexabromocyclododecane stereoisomers (HBCDs) are used as flame retardants in thermal insulation building materials, upholstery textiles, and electronics. Since 2004, the production and use of pentamix- and octamix- polybrominated diphenyl ethers (PBDEs) have been restricted in Europe. HBCDs might be used as an alternative for PBDEs, and are the second highest-volume brominated flame retardants (BFRs) used world wide, after tetrabromobisphenol A. Because of the widespread use and the physical and chemical stability, HBCDs are now ubiquitous contaminants in the environment. In Nagoya city, HBCDs were frequently measured in environment. It is required to reveal their environmental distribution, behavior and time trends.
Here we report the concentrations of HBCDs in water, sediment, and biological samples, and analyze the biomagnification potential, the stereoisomer profiles, and the time trends.
Analysis was undertaken by liquid chromatography/ tandem mass spectrometry (LC/MS/MS) on a diastereoisomer basis. Ninety-three samples (22 of river and sea water samples, 27 of sediment samples, 24 of fish and shellfish samples, and 20 of breast milk samples) in Nagoya city were measured.
HBCDs were detected in 18 water samples, of which concentrations of total isomers ranged from 0.4 to 280 ng/L. γ-HBCD dominated over the other isomers in water samples. Similarly, sediment samples were dominated by γ-HBCD, which was detected in all samples, ranged from 0.2 to 27 ng/g-dry. Time trend analysis by boring sediment core samples in Nagoya port showed a remarkable increase in HBCD concentrations since about 2000. This might be reflected by changing use of HBCDs from PBDEs according to regulation of EU on the production and use of two PBDEs.
In fish and shellfish samples, the maximum HBCDs concentration was about 5-10 times that reported in earlier Japanese studies. α-HBCD was the predominant isomer of HBCDs and was found in all the samples in levels from 24 to 6300 ng/g-lipid. On the other hand, γ-HBCD was predominant in some samples, and the isomer patterns of HBCDs were similar to those in industrial products. Samples in which γ-HBCD concentrations were higher might be collected near the source.
In breast milk samples, α-HBCD was also predominant in HBCDs and was found in all samples in concentrations from 1.5 to 13 ng/g-lipid. The concentrations of HBCDs in the samples of the same person decreased from 7 to 1.5 ng/g-lipid during 9 days to 300 days of post parturition.

PTh-136
13:30 – 14:40

LC/MS/MS determination of hair-dye ingredients in products, water and urine:estimation of human exposure and environmental release.

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Keywords:
exposure, environment, release, hair-dye

Novel aspects:
Human exposure and environmental release of hair-dye ingredients were examined by developing measurement method by LC/MS/MS.

Abstract:
Introduction : Over 5,000 different chemicals are used in hair-dye products, some of which are reported to be carcinogenic and/or allergic. Aminophenol, diaminotoluene, aminocresol and resorcinol are well used and hazardous ingredients of hair-dye. The authors developed an LC/MS/MS method for simultaneously determining the hair-dye ingredients and applied it to the estimation of human exposure and environmental release of the ingredients.

Methods :
（1）measurement of the chemicals in shampoo and rinse water
After coloring, the hair was shampooed with one push (16g) of shampoo bottle. Then the hair was rinsed with water（5 L）, which was collected in a plastic bucket. A hundred milliliter of the rinsed water was loaded to Solid Phase Extraction Cartridges (C18 and Oasis MCX plus were combined in series）. The elute with methanol（5 ml) from C18 and the elutes with methanol（5 ml）, ammonia/methanol solution（1 /19, 5 ml) from Oasis MCX plus were separately concentrated to near 0.5ml under nitrogen gas stream. The concentrates were diluted with acetonitrile, filtrated and prepared in 2 ml for LC/MS/MS analysis.
（2）measurement of the chemicals in urine.
Within 24 hours after coloring the hair, all the urine were separately collected in glass bottles and stored in cool box. Every ten milliliter of urine was diluted with 40ml of water, and applied to the SPE. Samples were prepared with similar way to above.

Predominant result :
（1）determination of hair-dye ingredients in shampoo and rinse water
For the SPE method development, five kinds of SPE cartridges were examined, i.e. Slim-J PRS, AC-2, NEXUS, HLB and MCX. Although good recovery was not obtained with any of them, MCX had a potential to collect hydrophilic hair-dye ingredients by connecting it with other SPE cartridge. It was the best in the recoveries to connect C18 cartridge upstream of MCX. With the series connection of C18 and MCX, the recoveries of 5-amino-o-cresol, 2,5-diamino-toluene, p-amino-phenol and resorcinol spiked in shampoo and rinse water had improved from 15.5% to 96%, from 39% to 76%, from 21% to 56%, and 0% to 28%, respectively. An effect of pH on recovery was examined, because the MCX performs the best under pH 3 &#65374 ; 4. But it was concluded that higher recovery efficiency was obtained under pH value of 9.5 than that of 3.
A shampoo and rinse water sample was analyzed at 0, 2, 4, 8 and 24 hours afterward. The examined hair-dyes were two kinds, i.e. black and golden. The ingredients of the dyes differ to each other ; 2,5-diamino-toluene and p-amino-phenol are contained in the black dye, and 5-amino-o-cresol, p-amino-phenol and resorcinol are in the golden. With progress of time, the concentration of every substance decreased. In particular p-amino-phenol which was contained in golden hair-dye showed remarkable decrease in concentration from 4 hours after the shampoo. And as for resorcinol, the rate of the decrease was much slower than that of others. Further study is required to pursue the persistence of resorcinol.

（2）determination of hair-dye ingredients in urine
It is still under examination. Because of many impurities in urine, more efficient sample preparation method is required. .

PTh-137
11:10 – 12:20

Qualitative analysis of waste leachate by using exact mass of LC/Q-ToFMS/MS

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Keywords:
LC/Q-ToFMS/MS, MetFrag, irregular dumping

Novel aspects:
The simultaneously fractionation method followed by LC/ToFMS/MS was developed. The exact masses of product ions and precursor ions were analyzed by using MetFrag.

Abstract:
Introduction :
Environmental pollution by chemical substances has been a serious problem. In our daily life the various types of chemical substances are used and wasted. The authors aim to develop a method comprehensively separating chemicals according to their properties and qualifying them with LC/Q-ToFMS/MS. Here we report a qualitative analysis of a leachate from irregular waste dumping area by a provisional qualification method with LC/Q-ToFMS/MS.

Methods :
To separate a variety of chemicals, a solid phase extraction (SPE) method using C18 cartridge followed with ion exchange cartridges (Oasis MAX, WAX, MCX, WCX) was developed. Chemicals collected in the SPE cartridges were fractionated with the way as follows ; Two liter of water sample was loaded into C-18. Substances collected in C18 were eluted with 10mL of methanol. The water passed through C18 was divided into four equal parts of 2 L. The every 500mL of C18 passed fraction was separately loaded into Oasis MCX, MAX, WCX and WAX. From the MCX and the WAX, collected substances were eluted with 10mL of methanol/ammonium solution. From the MAX and the WCX, collected chemicals were eluted with 10mL of methanol/formic acid solution. All the fractions were concentrated to 2 mL in volume and served for accurate mass measurement using LC/Q-ToFMS/MS.
The exact mass spectra were analyzed with 'MetFrag' (Leibniz-Institut für Pflanzenbiochemie) by ways as follows ; the elemental compositions of intact molecular ions were analyzed with the mass accuracy of better than 3 ppm. Then molecular structures that agree with the elemental composition were extracted from 'ChemSpider' (chemical structure database by UK in the 26 million structures) and the structures of which predicted fragments match the sample spectrum (with mass accuracy of better than 6 ppm) were chosen.. Chemicals in a leachate from irregularly dumped waste were qualified by the method written above.

Predominant result :
Hundreds or more candidates of chemicals were extracted by simply eliminating chemicals of which intact molecular ions didn't fit the mass spectra with the mass accuracy of better than 3 ppm. It could be possible to identify some chemicals from the sample mass spectra with the combination of the product ion analysis with mass accuracy of better than 6 ppm. In a leachate from irregular waste disposal site, pharmaceuticals, flame retardants, surfactants and other chemicals were found ; VALGANCICLOVIR (0.28ppm Antiviral agents) , Etodroxizin dimaleate (1.91ppm Sedative) . Phosphoricacid-tris- (2 -butoxyethyl) (0.75ppm) , Dimethylaminophenol (2.19ppm Resin hardener) and a polyethoxylate [-OCH_2CH_2-] surfactant were found. The LogP values of the substances above were ranged from-1.27 to 3.50.
The phosphorus-based flame retardant would be from industrial waste. The pharmaceuticals might be from a different source from industrial wastes. The presented method is provisional until it has been validated with various environmental samples.
With the presented SPE method, hydrophilic and hydrophobic substances could be simultaneously collected, fractionated and qualified by LC/Q-ToFMS/MS.

PTh-138
13:30 – 14:40

Investigation of Perfluorinated compounds in Osaka-bay over past three years

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Keywords:
Perfluorinated compounds, Osaka-bay, Environmental investigation, LC-MS/MS

Novel aspects:
Contamination of PFCs in Osaka-bay has been insufficient information.This research presented the characteristic distribution and the change of concentrations of PFCs in Osaka-bay.

Abstract:
Perfluorinated compounds (PFCs) have been widely used by various consumers and industrial products for over 50 years. PFCs are used for various products commencing with surface protectors in carpets, paper, packaging, and fire fighting foams. However, PFCs, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been found to be persistent, bioaccumulative, and toxicant Furthermore, many studies have been reported on global distribution of PFOS, PFOA and the homologues, such as perfluorinated carboxylic acids (PFCAs) and perfluorinated alkyl sulfonates (PFASs) . PFCs have been detected in human bloods, biota, sea water, and remote areas such as the Arctic.

In Japan, PFCs contamination, especially PFOA, in Keihanshin area (the area over Osaka prefecture, Kyoto prefecture, and Hyogo prefecture) were recognized by many studies. On the other hand, fluoro resin manufacturers and fluoro resin suppliers are reducing emissions and product content of PFOA, precursors, and higher homologues based on the USEPA's "2010/15 PFOA Stewardship Program ". Some companies are using PFCs of C 6 such as Perfluorohexanoic acid (PFHxA) as alternative materials. Composition of PFCs in the environment may change reflecting these activities. In addition, serum half-life in rats and monkey and some toxic properties of PFHxA was lower than PFOA. Therefore, it is considered that risk of PFHxA is smaller than PFOA.

In this study, to confirm the concentration and composition of PFCs in the coastal region of Osaka-bay, Japan, total of 12 PFCs, which were PFOA, PFOS and the homologues, were investigated. The coastal region of Osaka-bay is one of the main densely-populated and industrial areas in Japan. It seems that the concentrations of PFCs in the sea reflect emission from points and/or non-point source of the coastal land area. The sea water samples were collected in 2008, 2009, and 2010. The samples were stored in 4 ° C until analysis. The sample was extracted by a solid phase extraction cartridge. The loaded cartridge was eluted by Methanol. The eluted solution was concentrated to 1 mL. The final solution was carried out by using liquid chromatography (LC) -tandem mass spectrometer (MS/MS) . The analysis was conducted using negative electrospray ionization with multiple reaction monitoring.

PFHxA and PFOA were detected in all samples. Concentration of PFHxA ranged from 11 to 760 ng/L. Concentration of PFOA ranged from <MQL to 62 ng/L. Composition ratio of PFHxA was dominant in all samples. The concentrations in the coast had been reported few ng/L or few hundred ng/L by other studies. The concentrations of PFHxA and PFOA in this study were of similar value to other literatures. However, the concentration of PFCs in pacific, which had been considered that there is no influence from defined source, had been reported few pg/L or few ten pg/L. It seems that there is discharge of PFCs from the coast to the sea. Additionally, as for the characteristics of spatial distribution of PFCs, concentrations at closed-off section were high. PFCs might be discharged from closed-off section. Concentrations of PFHxA were increased by one order of magnitude from 2008 to 2009. It seems that amount of the discharge had mounted in around 2008.

PTh-139
11:10 – 12:20

Computational Chemistry Study on Negative Ion Chemical Ionization Mechanism of Peroxyacetyl Nitrate

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Keywords:
Electron capture chemical ionization, DFT, MOPAC, Photochemical oxidant

Novel aspects:
The fragmentation and ionization mechanisms of PAN was theoretically elucidated. This helps us to guess the major NICI/MS fragment ions of unidentified PANs.

Abstract:
Atmospheric photochemical reactions of non-methane hydrocarbons (NMHCs) and nitrogen oxides (NO_x) produce wide variety of peroxyacyl nitrates (PANs, RC (O) $OONO_2$) . They transport NO_x far downwind to affect the photochemistry in remote areas. PANs affect adversely on human, and their mutagenic activities are species-dependent. Recently, negative ion chemical ionization/ mass spectrometer (NICI/MS) was reported as a sensitive and selective detector for PANs. On the other hand, their exlosive and thermally unstable nature makes it difficult to synthesize their authentic standards. In this study, the behaviors of peroxyacetyl nitrate (PAN, CH_3C (O) $OONO_2$) , the most common class of PANs, in the NICI/MS source was investigated by means of computational chemistry study to fully understand the production mechanism of the dominant ions by means of density functional theory and semi-empirical molecular orbital calculations.

The NICI process was initiated by the electron attachment on PAN. The resulting PNA^- seemed highly unstable with respect to the O-O bond dissociation. Besides, the O-N bond seemed also dissociable for PAN^- at the Frank-Condon state. The possible neutral products from the two dissociation channels were electrophilic, and therefore could capture additional electrons to ionize in the NICI/MS source. Accordingly, NO_3^-, NO_2^-, CH_3C (O) O^-, and CH_3C (O) OO^- were possible product ions during the NICI/MS analysis of PAN.

PTh-140 Analysis of inadvertent PCBs contained in consumer goods

13:30 – 14:40

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Keywords:
inadvertent PCB congener yellow pigment

Novel aspects:
Some PCB congeners showing irregular composition profile were found in some pigments, and becoming social concern. Analyses of inadvertent PCBs contained in consumer goods were carried out.

Abstract:
Although production and use of polychlorinated biphenyls (PCBs) were banned in early 70s in Japan, safe storage and disposal of PCBs are still social concern. In addition to that, "irregular " PCB congeners, showing different composition from PCB products, were found unexpectedly in wastewater (1) followed by in air and river water (2, 3), giving rise to another social and technical concern. The origin of such "irregular " congeners has been studied and elucidated (2); they are from polychlorobenzidine dyes and production process thereof and released to the environment finally.

Among 209 PCB congeners, 3,3'-dichlorobiphenyl (PCB 11) and 2,5,2',5'-tetrachlorobiphenyl (PCB 52) are the most typical inadvertent ones. These congeners in some organic pigments showed different composition profile from the one seen in ordinary PCB products. This means that some portion of PCBs found in the environment may be the byproducts of pigment. Little attention had been paid for them until recently, though US Code of Federal Regulations pointed out the unintentional formation of such byproducts in the yellow pigments in 1979 (4).

Based on such a background, Japanese Government placed an official request for emergency survey of PCB content as for several certain pigments against manufacturers and importers thereof in February 2012 (5). Several consecutive actions have been undertaken so far.

From a point of risk management, it is more important to know the exact congener composition than to know total PCB content, as every congener has its own toxicity varying from the top to the bottom. For such a precise purpose, powerful high resolution GC-MS is preferably used instead of conventional GC-ECD, because GC-ECD, often used for PCB determination in electrical condenser oil, cannot differentiate congener peaks.

Organic pigments have replaced traditional Cadmium Yellow aiming to avoid cadmium, and they are now used for wide variety of industrial products as well as daily consumer goods. It is quite rational to think "Do yellow retail products carry such congeners? " Some analytical results are available in the literature (6), however ordinary consumers may know very little about the material composition of consumer goods. That is why upstream compound maker may have it closed with secrecy reason. Therefore we are somehow anxious about yellow stuff, whether it contains unintentional PCB congeners or not.

Analyses of inadvertent PCBs contained in yellow consumer goods such as crayon, ink, paint, lacquer, adhesive tape and so on were carried out by utilizing capillary GC equipped with high resolution MS in this study. In comparison to the environmental samples such as air and water, sampling itself was easy as for consumer goods. However, it seemed to be difficult to construct typical one standard method so as to cover all samples we dealt. From an experimental viewpoint, clean-up protocol shall be modified case by case according to the property of test sample.

Analytical results obtained are to be presented in this paper.

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PTh-141
11:10 – 12:20

Method development for simultaneous analysis of hydroxylated polychlorinated biphenyl by GC-ECNI/MS in biota sample

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Keywords:
hydroxylated polychlorinated biphenyls, biota samples, GC-LRMS (ECNI) , isotope dilution

Novel aspects:
The present study is to develop the fast and high sensitive methods for the analysis of OH-PCBs encompassing a wide range of homologues in blood sample using GC-LRMS (ECNI) .

Abstract:
Halogenated phenolic compounds are known to be strongly retained in human and wildlife blood. Among those compounds, a large number of hydroxylated polychlorinated biphenyls (OH-PCBs) have earlier been reported in blood from humans, terrestrial and marine mammals.

Some analytical methods, which employed mainly gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) using electron ionization mode (EI) have already been applied for OH-PCBs analysis. Although GC-HRMS (EI) can provide high sensitive and selective data, the instruments are extremely expensive for routine and high through-put analysis and require advanced technical skills for operation. A gas chromatography coupled to low-resolution mass spectrometry (GC-LRMS) with electron capture negative ionization mode (ECNI) is widely used analytical instruments in environmental chemistry.

The objective of the present study is to develop the fast and high sensitive methods for the analysis of OH-PCBs encompassing a wide range of homologues in blood sample by isotope dilution method using GC-LRMS (ECNI) analysis.

In biota sample, extracts of human serum (2-3 g) and whole blood (10g) samples from human and wildlife were analyzed. Briefly, blood samples were denatured with 6 M HCl and 2-propanol, and extracted with 50 % methyl t-butyl ether (MTBE) / hexane. $^{13}C_{12}$-labeled PCBs as well as OH-PCBs were spiked as internal standards. The organic phase was partitioned into neutral (containing PCBs) and phenolic (containing OH-PCBs) fractions with KOH (1 M ethanol/H_2O, 1 : 1, v/v) . The alkaline phase (containing OH-PCBs) was acidified to pH 2 with sulfuric acid and re-extracted with MTBE/hexane. OH-PCBs fraction was passed through non-activated silica-gel column chromatography. OH-PCBs were derivatized with trimethylsilyldiazomethane. The derivatized solution was further cleaned up by using activated silica-gel column. Identification and quantification of MeO-PCBs were analyzed by GC-LRMS (ECNI) and compared to GC-HRMS (EI) .

Firstly, we compared the instrumental detection limit (IDL) of OH-PCBs in GC-LRMS (ECNI) and GC-HRMS (EI) . The IDL was defined as 3 times the standard deviation (SD) of 5 replicate injections of a low concentration standard solution of OH-PCBs (1 pg ml^{-1}) . The IDLs were 4 ´ OH-triCB29 (22 pg) , 4 ´ OH-tetraCB79 (14 pg) , 4 OH-pentaCB107 (0.65 pg) , 4 OH-hexaCB146 (1.6pg) , 4 OH-hexaCB159 (0.90 pg) , 4 ´ OH-hepta-CB172 (0.81 pg) ,4OH-heptaCB187 (0.57 pg) and 4 OH-octaCB201 (0.63 pg) by GC-ECNI-MS analysis, and compared to 4 ´ OH-triCB29 (0.82 pg) , 4 ´ OH-tetraCB79 (0.56 pg) , 4 OH-pentaCB107 (0.34 pg) , 4 OH-hexaCB146 (0.24pg) , 4 OH-hexaCB159 (0.23 pg) , 4 ´ OH-hepta-CB172 (0.40 pg) ,4OH-heptaCB187 (0.20 pg) and 4 OH-octaCB201 (0.52 pg) by GC-HRMS (EI) . The sensitivity of penta- to octa- OH-PCBs determined using the GC-LRMS (ECNI) was in agreement with the GC-HRMS (EI) method. However, sensitivities of GC-LRMS (ECNI) to tri- to tetra- OH-PCBs of GC-LRMS (ECNI) were lower than of GC-HRMS (EI) .

The recoveries of $^{13}C_{12}$ labeled OH-PCB inblood samples were 61-113 %. The method repeatability was in the range 6.7-10.4% and 0.6-2.5% relative standard deviation (RSD) for the GC-LRMS (ECNI) and GC-HRMS (EI) systems, respectively.

In conclusion, the GC-LRMS (ECNI) and GC-HRMS (EI) systems were found to be equally well suited for determination for OH-PCBs in biological samples at the pg-levels order of penta- to octa-OH-PCBs.

PTh-142 Suspended solid as a disturbance of PFOS analysis in case of wastewater

13:30 – 14:40

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Keywords:
PFOS SS surrogate recovery COP 4

Novel aspects:
Recovery of surrogate substance in PFOS analysis was investigated to improve current method, and the effect of chain length of perfluorinatedalkane on the recovery is to be discussed.

Abstract:
Perfluorooctane Sulfonate (PFOS) is one of the Perfluoroalkane Sulfonates (PFASs) and is also the terminal degradation product of many perfluoroalkyl compounds (PFCs) . Specific properties of PFCs such as water repellency, thermal stability, and surface tension lowering, made these compounds important both for commercial and technical aspects.

As a consequence, PFOS having several emission paths to the environment had been broadly spread out over 50 years. Recently PFOS was designated and restricted as persistent organic compounds (POPs) by the Stockholm Convention on POPs (1) .

In Japan, PFOS was specified to the first class chemical under the Chemical Substance Control Law followed by filing technical guideline for disposal of PFOS containing garbage in March 2011 (2) . Several kinds of standard analytical method using LC-MS/MS (2-4) had been proposed for surface water prior to that.

Many papers pointed out the technical difficulties arising from the contamination owing to fluorine resin used for connectors or tubing material in the apparatus, even when clean water was analyzed (5) .

Other than contamination, we encountered different difficulties when we applied current analytical methods to the wastewater in the beginning of our study. We experienced low recovery of surrogate substance that was added to the sample to ensure the precision. Low surrogate recovery did not meet the requirements prescribed in the current analytical standards designed for surface water.

We found the reason for the difficulties. On one hand, it is known among the lab analysts that concentration of PFOS is prone to be decreased probably owing to the adsorption toward labware. But on the other hand, little attention was paid for the adsorption onto suspended solid (SS) in case of surface water, in spite of numerous efforts to develop sampling and analytical methods for PFOS, since SS content in surface water is generally enough low (6) .

Development of harmless disposal method undergoes, analysis of wastewater we concern shall be required. Proper care should be taken for adsorption on SS for the analysis of water containing high SS.

Among elution conditions of PFOS, eluent composition was intensively investigated, and in addition we found both quality and quantity of SS affected on surrogate recovery, leading to an improvement toward current method. We would suggest the importance of SS remained on the filter upon analytical results.

The last portion of this study relates to the effect of perfluoroalkane chain length on the recovery, and results are to be discussed.

Acknowledgements
The authors thank Mr. Shusuke Takemine of Hyogo Prefectural Institute of Environmental Sciences for helpful discussions about PFOS nature.

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PTh-143
11:10 – 12:20

Analysis of Metabolites emitted by Soil-Derived Fungi using Ion Mobility Spectrometry based on GC/MS Data Analysis

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Keywords:
MVOC；Metabolite；Ion Mobility Spectrometrty；Mass Spectrometry；Cultural Properties

Novel aspects:
The microbial volatile organic compounds emitted from fungi may be applicable to a notification of fungal growth in the environment in order to preserve cultural properties using MS or IMS.

Abstract:
Contaminations of fungi were found on the mural paintings in the famous Takamatsuzuka tumulus in Japan [1]. Fungi often cause serious damage to cultural properties. The purpose of our work is to detect fungal growth by monitoring Microbial Volatile Organic Compounds (MVOCs) emitted from fungi at cultural sites.
Ion Mobility Spectrometer (IMS) is suitable for on location measurements because it is portable. Furthermore, it is a powerful tool to simultaneously detect multiple compounds. However IMS alone cannot be used to identify these MVOCs. In order to identify these compounds, we also must use GC/MS. In this study, metabolites emitted by soil-derived fungi were analyzed using both Ion Mobility Spectrometry and Mass Spectrometry.
Alcohols, aldehydes and ketones were found as MVOCs in most of *Aspergillusfumigatus*, *Aspergillus nidulans*, *Fusarium solani* and *Penicillium paneum*. On the other hand, sesquiterpenes were found in only specific fungi. Because each fungal strain had a characteristic compound (sesquiterpene), these compounds are useful in identifying their respective fungi. As the number of spores increased with the fungal cultivation period, it was found that the amount of these MVOCs, ketones, aldehydes and alcohols also increased. Therefore, 3-octanone is suited to be an indicator of the size of fungi (fungal amounts) because of the positive correlation found between the number of spores and the amount of the compound. On the other hand, sesquiterpenes showed a peak of MVOCs at a particular period right before spore reproduction and therefore are useful to identify both fungal species and their reproduction periods [2].
Ion Mobility Spectra of volatile metabolites emitted from *A. nidulans* were measured using the IMS-MINI Ion Mobility Spectrometer (I.U.T. GmbH, Germany). In the IMS driftgram of MVOCs from *A. nidulans,* a large peak appeared at 6.36ms which correspond to "Reaction Ion Peak" (RIP). From the assignment based on the comparison with GC/MS data analysis of MVOCs from *A. nidulans* cultivated under the same condition as samples measured by IMS; it was found that relatively smaller peaks appeared at 7.69, 8.31 and 9.68ms were assigned to phenyl acetaldehyde, 3-octanone and bisabolene, respectively. A peak at 7.50 ms corresponds to 2-octen-1-ol and 1-octen-3-ol. The IMSdriftgram of volatile metabolites emitted *P. paneum* showed the peak corresponding to beta-Caryophyllene. This compound is one of sesquiterpenes and an unique MVOC for *P. paneum.* Therefore, it was concluded that *P. paneum* can be monitored using only a driftgram of MVOC from *P. paneum*.
The IMS drift time for these compounds was also calculated using Monte Carlo simulations.
The calculated drift time was consist with the experiments.

References

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PTh-144

13:30 – 14:40

Measurement of brominated flame retardants in the environment near factories by LC/MS/MS.

Itaru Oue, Shigeru Suzuki

CHUBU University,Aichi,Japan

Keywords:

BFRs, road-dusts, LC/MS/MS, factories

Novel aspects:

BFRs in river waters, sediments, effluents and road-dusts near factories were determined by LC/MS/MS.

Abstract:

Introduction : Brominated flame retardants (BFRs) are widely used in plastics, rubbers, textiles and other products for fire protection, and are discharged into the environment through air emissions, water discharges, wastes and end products. In the previous studies, the authors found unexpected contaminations with BFRs in not only plastic products such as baby toys, chew toys and household products, but in road dust around the non BFR plastic moulding factories. Here we present a research predominantly taken place around factories by measuring BFRs in effluents, river waters, sediments and road dusts.

Method : Waters, sediments and road dusts were collected in rivers, effluent water ways or road sides near the factories. Water samples were added with formic acid (500 μL to every 500mL of water) and stored in cool and dark place. Sediment samples were collected in glass bottles and stored in freezer. Road dusts were sampled in glass bottles and stored in cool and dark place. Water sample was filtered and the filtrate was extracted by solid phase extraction (SPE) with C18 sorbent (Inert Sep C18, 500 mg) which was conditioned with methanol (5 mL) and distilled water (5 mL) in the order. The sample loaded cartridge was cleaned by flushing with 50%methanol (1 mL), and then the target substances ware eluted from the cartridge with methanol (5 ml) and acetone (5 mL). The residual suspended solids on the filter and the glassware used for sample preparations were rinsed in the order with acetone (5 mL) and dichloromethane (5 mL), which were added to the C18 extract. The extract was concentrated with nitrogen, and the solvents were exchanged to acetonitrile followed by LC/MS/MS (with API3000). Sediments and road dusts were extracted in the order with acetone (5 mL, two times) and dichloromethane (5 mL, two times). The extract were concentrated and cleaned up by SPE with C18, and measured by LC/MS/MS.

Predominant result : : The method recoveries were studied by analyzing five replicates of samples spiked with Hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), tribromophenol (TBP) and bisphenol A (BPA). Recoveries of HBCD, TBBPA, TBP and BPA were 63% with relative standard deviation (RSD) of 8 %, 102% with RSD of 17%, 98% with RSD of 12% and 106% with RSD of 4 %, respectively. It would be the reason for the lower recovery of HBCD (around 60%) that the HBCD is extremely hydrophobic substance, which might be adsorbed on the surface of glassware, SPE adsorbents and other hydrophobic materials.

In research of a dyeing processing factory, water concentrations of TBP and BPA were 1.3-6.3ppt and 9.0-950ppt, respectively. On the other hand, those of HBCD were lower than limit of detection in most samples excepting in the samples of the effluent and near the effluent discharge point.in a river. All other results will be presented in the poster.

PTh-145
11:10 – 12:20

Determination of sulfonamides and tetracyclines in livestock wastewater using hybrid ion trap - time of flight mass spectrometer

Youngmin Hong[1], Hyunook Kim[2]

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Keywords:
Sulfonamide, Tetracycline, Ion Trap, Time of Flight, Wastewater

Novel aspects:
Analytical method of sulfonamides and tetracyclines using hybrid ion trap - time of flight MS was described. Sample extraction was carried out with a HLB and a MCX cartridges.

Abstract:
Veterinary drugs are widely used in large quantities for treatment of disease or to promote animal growth. Nowadays, veterinary drugs are recognized as not only medicines but also as 'Newly Emerging Contaminants' in the environment. The aim of this study is to monitor organic compounds in aqueous samples using Liquid chromatograph coupled to hybrid ion trap - time of flight mass spectrometer. The analyzed compounds were 3 sulfonamides (i.e., sulfathiazole, sulfamethazine, and sulfamethoxazole) , and 2 tetracyclines (i.e., oxytetracycline and chlortetracycline) in samples from a livestock wastewater treatment plant (WWTP) . Sample extraction was carried out with a hydrophilic-lipophilic balance and a mixed-mode cation exchange solid phase extraction cartridges. Average recoveries of sulfonamides at fortification levels from 1.0 and 4.0 $\mu g\ L^{-1}$ in effluent of a local domestic WWTP were 73-95 % and 89-104 %, respectively, while tetracyclines at 0.4 and 4.0 $\mu g\ L^{-1}$ were 76-104 % and 101-107 %, respectively. The method detection limits were 22.8, 23.0, 25.8, 23.6, and 9.8 ng L^{-1} for sulfathiazole, sulfamethazine, sulfamethoxazole, oxytetracycline, and chlortetracycline, respectively. The maximum concentration of sulfonamide and tetracycline residues detected in samples were 49.5 and 4.1 $\mu g\ L^{-1}$, respectively. The developed method could be applied successfully to quantitate residual sulfonamides and tetracyclines in animal wastewater. The analysis of the samples showed that more than 90% of target pharmaceuticals were removed in the animal WWTP consisting of a biological process, a UF membrane, and a coagulation process.

PTh-146
13:30 – 14:40

Dechlorane Plus, a highly chlorinated flame retardant in Japanese environment samples.

Takanori Sakiyama[1], Takeshi Nakano[2]

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Keywords:
Dechlorane Plus, Japan, Environment

Novel aspects:
Dechlorane Plus was determined in environment samples for the first time in Japan.

Abstract:
Dechlorane Plus (DP) is a highly chlorinated flame retardant, was developed to replace Mirex (Dechlorane) that was banned in the 1970s. It is manufactured about 450 ton per year by Occidental Chemical Corporation (OxyChem) in the United States. In 2006, the first of sightings of this chlorinated flame retardant were in the Great Lakes region, and the investigation reports are increasing rapidly recently in the world, mainly in North America. More recently, a newly DP production facility was discovered in China. It is estimated that Chinese capacity of DP production has reached 2,000 ton in 2006.

However, to our knowledge, no data for levels of DP in environmental samples were available in Japan. Therefore, we measured DP by using the GC / high resolution MS in EI mode, and identified this compound in the environmental samples collected from Japanese urban area. DP was detected in house dust, deposit of the window frame, road sediment, garden soil and sediment samples at concentrations ranging from 2.9-42ng/g-dry,240-270ng/g-dry, 74-150ng/g-dry, 1.7ng/g-dry, 17-140ng/g-dry, respectively. Further, we showed the mean of *anti*-DP fractional abundance (f_{anti}) value in these Japanese samples was 0.65, 0.83, 0.80, 0.81, 0.81, respectively. This compound had not been identified in the environment of Japan before our report. In this conference, we will indicate the detailed additional data of DP in surface sediment samples of the urban river in Japan.

PTh-147
11:10 – 12:20

Multi-residue method for rapid screening of veterinary drugs in muscle matrices by UHPLC-MS/MS

Youngmin Hong[1], Sujeong Park[2], Insun Lee[1], Minhye Lee[1]

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Keywords:
Multi-residue screening, Veterinary drugs, UHPLC-MS/MS

Novel aspects:
A rapid multi-residue screening method was developed for the 180 veterinary drugs and their metabolites using a UHPLC-MS/MS.

Abstract:
A rapid multi-residue screening method was developed for the simultaneous analysis of about 180 veterinary drugs and their metabolites using a ultra-high-performance liquid chromatograph coupled with tandem mass spectrometer (UHPLC-MS/MS) . The screened veterinary drugs belonged to amphenicols, anthelmintics, benzimidazoles, β-lactams, coccidiostats, ionophores, macrolides, non-steroidal anti-inflammatory agents, quinolones, sulfonamides, tetracyclines and tranquilizers. The drugs were extracted from bovine, porcine, and chicken muscle samples with acetonitrile water (4 : 1, v/v) containing 2 mM ammonium formate, and the extracts were applied to the dispersive solid phase extraction and n-hexane clean-up procedure. Reverse-phase LC separation was accomplished on a C_{18} column and gradient elution with a mobile phase consisting of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The method had a chromatographic total run time of 5 min. The separated compounds were detected with a Shimadzu LCMS-8030 tandem quadrupole mass spectrometer operating with an electrospray ion source (ESI) in positive and negative switching mode by applying a time scheduled multiple reaction monitoring of 2 or 3 transitions. The developed method was validated according to the EU Commission Decision 2002/657/EEC for a quantitative screening method. All the validation data, such as the mean accuracy, the repeatability, the within-laboratory reproducibility and the detection capability, CC_β accuracy were within the required limits.

PTh-148
13:30 – 14:40

Development of LC-MS/MS method to monitor pharmaceuticals in environmental wastewater

Youngmin Hong, Insun Lee, Minhye Lee

Dongil Shimadzu, Seoul, Korea

Keywords:
Pharmaceutical, Wastewater, UHPLC-MS/MS

Novel aspects:
An analytical method for screening and confirming of 30 pharmaceuticals in water and wastewater samples was developed using a UHPLC-MS/MS.

Abstract:
A rapid and reliable LC-MS/MS method was developed for the simultaneous identification, confirmation and quantification of 30 pharmaceuticals in water and wastewater samples. Two distinct chromatographic conditions were used according to the polarity. The method used sub two micron size C18 and hydrophilic interaction chromatography columns. The separated compounds were detected with a Shimadzu LCMS-8030 tandem quadrupole mass spectrometer operating with an electrospray ion source (ESI) in positive and negative switching mode by applying a time scheduled multiple reaction monitoring of 2 or 3 transitions. The analytical performance of the method was evaluated for sample collected from a local domestic wastewater treatment plant. Sample extraction was carried out with a hydrophilic-lipophilic balance and a mixed-mode cation exchange solid phase extraction cartridges. To minimize the matrix effect, matrix-matched standards were analyzed in a wastewater effluent. Under the study, all compounds gave good sensitivity over a level of ng L^{-1} with r^2 values of 0.99 or greater. Average recoveries of all compounds at 10 and 1,000 ng L^{-1} were about 54-105%.

PTh-149 Analysis of Ultraviolet Absorbers in Urine Samples by Functionalized Nanomaterial-assisted Electrospray Mass Spectrometry

11:10 – 12:20

Tzung-Jie Yang, Maw-Rong Lee

National Chung Hsing university, Taichung, Taiwan

Keywords:

Ultraviolet Absorbers；Functionalized Nanomaterial-assisted Electrospray Mass Spectrometry

Novel aspects:

The nanomaterial-assisted electrospray ionization offers a high specific and high throughput screening for trace analysis.

Abstract:

Functionalized nanomaterials assisted electrospray ionization for ananlysis of ultraviolet absorbers in urine was developed in this study. The electrospray was performed with high voltage on a laboratory-made screen-printed plate with coating a layer of silver or graphite. The ultraviolet absorbers studies in this study are including 3-(4-methylbenzylidene)-camphor (4-MBC), 2-ethyl-4-(dimethylamino) benzoate (*OD*-PABA) and 2-hydroxy-4-(octyloxy)-benzophenone (BP-12). The analytes were extracted with nanomaterials that included iron oxide-coated silicon dioxide nanoparticles, multiwall carbon nanotubes (MWCNTs) and graphene oxide (GOx) was dipped on the screen-printed substrate to evaluate the effect on the ionization efficiency. After extraction, the extract or nanomaterial without doing any desorption. From the results, the MWCNTs can enhance the intensity of the signal detected and short the analytical time. This technique demonstrates that the nanoparticles assisted electrospray ionization offers a high specific and fast screening in trace analysis.

PTh-150

13:30 – 14:40

Determination of diuretics in urine using immobilized-multiwalled carbon nanotubes hollow fiber liquid-phase microextraction combined with liquid chromatography-mass spectrometry

Tse-Tsung Ho, Maw-Rong Lee

National Chung Hsing university, Taichung, Taiwan

Keywords:

Diuretics ; multiwalled carbon nanotubes ; hollow fiber liquid-phase microextraction ; liquid chromatography-mass spectrometry

Novel aspects:

The laboratory-made immobilized-multiwalled carbon nanotubes (I-MWCNTs) in hollow fiber liquid-phase microextraction (HF-LPME) combined with LC-MS for analyzing diuretics in urine.

Abstract:

Diuretics, which increase urine flow from the kidneys, are used for treatment of heart conditions, liver, kidney and lung disease, generally to reduce salt or water retention. The Medical Commission of the International Olympic Committee (IOC) included diuretics as a banned substance since 1986. This study was to evaluate a sample treatment technique using immobilized-multiwalled carbon nanotubes (I-MWCNTs) in hollow fiber liquid-phase microextraction (HF-LPME) combined with liquid chromatography-mass spectrometry for diuretics analysis in urine samples. The optimal conditions of sample extraction and mass spectrometry have been studied. The detection limits for the diuretics studied were found to be in the range of 0.19 to 0.96 ng/mL with the relative standard deviation (RSD) below 11.1%. No carryover effect was found, and every laboratory-made I-MWCNTs HF-LPME could be reused for extraction up to 50 times with recovery efficiency above 85%. The I-MWCNTs HF-LPME has been proven effectively for increasing extraction efficiency and reducing matrix interference from urine. The method developed offers not only very high sensitivity for determination of trace diuretics in urine, but also reduced extraction costs for having a long use times of the I-MWCNTs HF. The method is recommended for determination of trace diuretics in urine for its elegance, simplicity and high sensitivity even in the presence of high levels of interference.

PTh-151
11:10 – 12:20

In-line preconcentration capillary electrophoresis-electrospray ionization-mass spectrometry for the analysis of haloacetic acids in tap water

Sih-Hua Hung, Guor-Rong Her

National Taiwan University, Taipei, Taiwan(R.O.C)

Keywords:
haloacetic acids, in-line preconcentration, CE-MS, tap water

Novel aspects:
A simple and sensitive FASI-CE-ESI-MS/MS method was developed for in-line monitoring haloacetic acids in tap water.

Abstract:
Haloacetic acids (HAAs) belong to an important class of disinfection byproducts that are being regulated. A strategy based on in-line preconcentration CE-ESI-MS/MS was developed for determination of haloacetic acids (HAAs) in tap water. The field amplified sample injection (FASI) technique was used for in-line preconcentration of five HAAs including monoacetic acid (MCAA) , dichloroacetic acid (DCAA) , trichloroacetic acid (TCAA) , dibromoacetic acid (DBAA) and monobromoacetic acid (MBAA) . In FASI-CE-MS/MS analysis, 50% MeOH containing 2.5%ammonium acetate (pH 3.5) was used as background electrolyte and sheath liquid. Low sheath flow interface was used to preserve separation condition and to stabilize electrospray ionization process. With 30 second hydrodynamic injection of a water plug followed by 20 second electrokinetic injection of the sample, FASI enrichment factors in a range of 1000-3000 were obtained. Detection limits of HAAs were in a range of 0.01-0.1 μgL^{-1}. In tap water analysis, three HAAs including TCAA, DCAA and MBAA were detected at a concentration about 0.1- 1 ppb, 0.1- 1 ppb and 0 -0.1ppb, respectively. The feasibility of FASI-CE-ESI-MS/MS in real time analysis is demonstrated by continuous monitoring HAAs in tap water.

PTh-152
13:30 – 14:40

Effects of silver on Chlamydomonas reinhardtii; insights from proteome analysis and physiological endpoints

Marc J Suter, Smitha Pillai, Holger Nestler, Rene Schoenenberger, Renata Behra, Kristin Schirmer

Eawag, Dubendorf, Switzerland

Keywords:
proteomics, silver, Chlamydomonas reinhardtii, ecotoxicology

Novel aspects:
systems biology view of silver-induced stress response in green algae. Linking of molecular responses to physiological effects

Abstract:
Classic ecotoxicology has focused upon a bottom-up approach to understand stressor effects in which a few genes, proteins, or biochemical reactions are studied at a time. The invention of new technologies in the last decade has enabled the analysis of whole transcriptome (gene transcripts) , proteome (proteins) and small cellular molecules (metabolite profiling) resulting in whole system approaches. By complementing the traditional approach with the system biology it is possible to define the genetic, protein, and biochemical reactions as integrated and interacting networks of anorganism. We use such a systems biology approach to understand the biological responses of *Chlamydomonas reinhardtii* to ionic silver, which is toxic to a variety of aquatic organisms in the nanomolar range. Specifically, the proteome profiling by Multidimensional Protein Identification Technology (MudPIT) allows determination of differential expression of proteins.
MudPIT analysis of *C.reinhardtii* exposed to silver allowed discrete identification of roughly 2500 proteins in each sample with false discovery rate set to 2 %, representing major cellular processes. Enrichment analysis showed significant regulation of several biological pathways. Key among them were photosynthesis, ATP synthesis and tetrapyrolle synthesis with all being severely down-regulated. Differently, some pathways that were up-regulated were the lipid synthesis, oxidative stress response, proteolysis and cell wall synthesis. Our results provide the first insights into the mechanisms of toxicity of ionic silver. Silver is taken up into the cells via active metal transporters and inhibits key proteins involved in photosynthesis and ATP synthesis. Silver also induces oxidative stress as deduced from the induction of oxidative stress response proteins such as GPXH. The up-regulation of lipid synthesis also indicates an autophagy response. Importantly, we could link the changes at the proteome to the physiological state of the algae on exposure to silver.

PTh-154 on-site analysis of gases emitted from soils using MULTUM-S II

13:30 – 14:40

Takahiro Anan[1], Shuichi Shimma[2], Yo Toma[3], Yasuyuki Hashidoko[4], Ryusuke Hatano[4], Morio Ishihara[1], Jun Aoki[1], Michisato Toyoda[1]

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Keywords:
on-site analysis, N 2 O

Novel aspects:
We detected N 2 O in the atmospheric concentration level (314ppb) by using the MULTUM-S II within one minute.

Abstract:
Recently, miniature mass spectrometers which can be used for on-site analysis have been designed and developed. These instruments are expected to have widespread applications. In our laboratory, a miniaturized multi-turn time-of-flight mass spectrometer "MULTUM-S II " was designed and constructed. Although the size of newly developed "MULTUM-S II " was 45 cm x 25 cm x 64 cm, it has capability of high mass resolution of more than 30, 000.

We attempted to carry out real-time monitoring of the microbial production in the soil and consumption of gaseous compounds (N_2O, CO_2, CO and CH_4) by using this MULTUM-S II. In this study, we attempted to monitor of N_2O concentration in real-time. N_2O is known as one of the important greenhouse gases, and its warming effect is 298 times higher than that of CO_2. Atmospheric N_2O concentration level is 314ppb and increases to several hundred ppm by bacterial activities. N_2O is mainly produced by N fertilizer in agricultural soils. Therefore, on-site high performance N_2O analyzer has been required to elucidate generating mechanism and estimate reduction procedure of generated N_2O from agricultural soils. However, N_2O is measured by using a gas chromatography (GC) with an electron capture detector (ECD) so that it is difficult to apply GC/ECD on the field due to the radioisotope (^{63}Ni) in the detector. On the other hand, we can measure N_2O in the field by GC/MS. In this method, it takes about ten minutes to separate CO_2 and N_2O in a separation column. If we could separate CO_2 and N_2O by using a high-mass resolution mass spectrometer, the measurement time would be reduced significantly. However, required mass resolution which is to separate CO_2 and N_2O doublet is larger than 8, 000 due to the mass difference is 0.0113 u. Therefore, conventional miniaturized instrument can't be separate the doublet. MULTUM-S II can provide high mass resolution in the compact size. Therefore, we attempted to separate CO_2 and N_2O doublet. In this experiment, we used 5 m PLOT column to separate N_2O, CO_2 and main components of air (N_2 and O_2) . When large amounts of N_2 and O_2 are injected into the ion source at the same time, low amount N_2O signal is suppressed. Using this procedure, we detected N_2O in the atmospheric concentration level (314ppb) . This result was significant increase compared to direct sample injection. Furthermore, the measurement time was within one minute. We also developed an automatic sampler to inject these gases into MULTUM-S II in one minute intervals for automatic real-time monitoring. As a result, real-time monitoring of atmospheric N_2O concentration and its variation of emission from soils can be achieved by the combination of the automatic sampler and the MULTUM-S II .

PTh-155
11:10 – 12:20

Level of Dechlorane Plus in ambient air and development of monitoring method

Koichiro MATSUMOTO[1], Yuko ODASHIMA[1], Yuji KASHIMA[1], Takanori SAKIYAMA[2], Takeshi NAKANO[3]

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Keywords:
Dechlorane Plus, ambient air, NCI

Novel aspects:
Dechlorane plus were determined in air samples for the first time in Japan.

Abstract:
A chlorinated flame retardant, Dechlorane Plus (DP) (*syn*-, *anti*-) , was detected and identified in ambient air from Japanese urban area. This study shows that DP was detected in air samples in Japan for the first time. In a moment of this research, we investigated the mode of ionization using GC/MS availability. GC/MS is commonly applying to determine DP with negative chemical ionization (NCI) mode at low resolution (LR) . DP was also determined magnetic sector-type MS with electron impact ionization (EI) mode at high resolution (HR) . R^2 for calibration curves on DP (*syn*-, *anti*-) were over 0.9999 of LR-NCI and HR-EI method. Instrument Detection Limits (IDL) of both methods for DP (*syn*-, *anti*-) were 0.20 pg/m^3, 0.23 pg/m^3 and 0.34 pg/m^3, 0.36 pg/m^3 respectively. LR-NCI method was S/N = 50 at 0.1 ng/mL standard solution that approximately four times greater than HR-EI method. Result of compared both methods, LR-NCI method was applying in this study.
Air samples were collected on the rooftop of Japan Environmental Sanitation Center. Samples were collected 24 hours and repeated 4 times. All samplings were implemented duplicate. Result of measurement by GC/MS (LR-NCI) , DP air concentration ranged from 1.9 to 21 pg/m^3. Recoveries of ^{13}C labeled DP were more than 90%. The *syn*- and *anti*- ratio were read between 0.24 and 0.48. All duplicates were considerably fit in each sample. DP concentrations were higher than Mirex to compare with Environmental Survey and Monitoring of Chemicals was implemented by Ministry of the Environment Japan.

PTh-156
13:30 – 14:40

Determination of hydroxylated polycyclic aromatic hydrocarbons in mariner's urine by high performance liquid chromatography-tandem mass spectrometry

Tetsuya Hirai[1,2], Hiroaki Kinoshita[1], Hideo Okamura[2], Yoshiji Yano[2], Takeshi Nakano[2]

[1]Otsuka Pharmaceutical Co., Tokushima, Japan, [2]Graduate School of Maritime Sciences Kobe University, Kobe, Japan

Keywords:
Polycyclic aromatic hydrocarbons, Urine, Human exposure, LC/MS/MS, metabolite

Novel aspects:
We assess the potential health risks posed by exhaust gas from ship and obtain a better understanding of the occupational hazards connected with PAHs exposure.

Abstract:
Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals released into the air during the incomplete burning of fossil fuels such as gasoline and other organic substances

Some PAHs are reasonable anticipated to be human carcinogens. The carcinogenic risk to humans among 16 PAHs is classified as probable human carcinogens by the International Agency of Research on Cancer in 2010. PAHs are also known to have endocrine disrupting activity. PAHs are absorbed into the human body through the skin, lungs and gastrointestinal tract and are then metabolized to their hydroxylated PAHs (OHPAHs) and finally excreted in urine. In this study, a high performance liquid chromatography-tandem mass spectrometry method has been developed for the simultaneous quantification of six urinary OHPAHs, including 1-hydroxynaphthalene (1-OHNap), 2-hydroxynaphthalene (2-OHNap), 1-hydroxyphenanthrene (1-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe) and 1-hydroxypyrene (1-OHPyr) in human urine. Deuterated 3-OHPhe-d 9 and 1-OHPyr-d 9 were used for the quantification of the analyte as internal standards. Considerable amounts of PAHs are present in the workplace. PAHs exposure is reported high in coke plants, aluminium work and paving work. In order to assess the potential health risks posed by exhaust gas from ship and obtain a better understanding of the occupational hazards connected with PAHs exposure, the concentration of OHPAHs in urine collected from mariners has been analyzed.

The urine sample treatment involved enzymatic hydrolysis of glucuronide and sulfate conjugates followed by solid-phase extraction using Sep-Pak C_{18} cartridge for LC/MS/MS analysis. The LC/MS/MS system consisted of an Agilent 1260 infinity series (Agilent Technologies, Santa Clara, CA, USA) and QTRP 5500 mass spectrometer (AB SCIEX, Framingham, MA, USA). The analyte and ISTD were separated from interference peaks on an Ascentis Express C18 column (2.1mmID × 100mm, 2.7μm particle size ; SIGMA-ALDRICH Co., St. Louis, MO, USA). The mass spectrometer was operated under multiple reaction monitoring (MRM) negative mode for the ion transitions m/z 143 →115 (OHNap), m/z 193 →193 (OHPhe), m/z 202 →202 (OHPhe-d 9), m/z 217 → 189 (OHPyr) and m/z 226 → 198 (OHPyr-d 9).

This method was applied to the analysis of OH-PAHs in 29 urine specimens (11 engineers and 18 other crews) collected from the crews on a ship. The average concentrations of urinary 1-OHNap, 2-OHNap, 1-OHPhe, 3-OHPhe, 4-OHPhe and 1-OHPyr were 1.6, 2.2, 0.2, 0.2,0.1 and 0.2 μg/g creatinine in engineers and 0.5, 1.8, 0.2, 0.1, 0.1 and 0.1 μg/g creatinine in other crews, respectively. The method can be used to evaluate occupational exposure to PAHs.

PTh-157 Thermal solid phase extraction for GC-MS analysis of complex samples

11:10 – 12:20

Vivi Kofoed-Soerensen, Asger W Noergaard, Peder Wolkoff, Per Axel Clausen

National Research Centre for the Working Environment (NRCWE)

Keywords:
GC-MS, thermal desorption, solid phase extraction, sample preparation

Novel aspects:
Thermal solid phase extraction (TSPE) is a fast and simple sample preparation for elimination of unwanted matrix compounds in GC-MS analysis of VOC and SVOC in complex matrices.

Abstract:

Introduction

Pristine samples or sample extracts often contain matrix compounds and/or solvents, which are incompatible with gas chromatographic analysis. Thus, an often complicated and time-consuming sample preparation with potential loss of analytes is needed before analysis. Here we present thermal solid phase extraction (TSPE) as a simple and fast approach based on thermal desorption (TD) for analysis of samples in complex matrixes. Samples are simply injected onto Tenax TA adsorbent tube and solvents (e.g. methanol, ethanol or water) are subsequently removed by purging with helium at ambient temperature. VOCs and SVOCs with boiling points up to ca. 400 °C are released during the following thermal desorption whereas larger and non-volatile compounds (e.g. sugars, proteins, fat, salts etc.) are retained by the adsorbent. Since TSPE may also be used for concentration of trace level samples by large volume injection onto the adsorbent, it can replace the following sample preparation steps :

1) Removal of unwanted matrix compounds that deteriorate the analysis
2) Solvent change to a suitable GC solvent
3) Up-concentration (e.g. by evaporation) of analytes before GC-MS analysis

Here TSPE has successfully been applied to the analysis of whisky and extracts of floor dust and tattoo inks.

Experimental

Single malt whiskies or methanol extracts of floor dust and tattoo inks were injected into stainless steel tubes containing Tenax TA adsorbent. The injections were followed by purging with He (60 ml/min for 3-5 min). Injection volumes were 1-50 µl. A thermal desorber (Perkin-Elmer ATD 400) was connected to a GC-MS system (Perkin-Elmer Autosystem XL GC/TurboMass MS or Varian CP 3800 GC/1200 MS). The Tenax TA tubes were desorbed for 20 min at 250 °C, using a He flow of 50 mL/min, and a cold trap temperature of -30 °C. The cold trap was narrow bore and packed with Tenax TA for VOCs and empty for SVOCs. Flash heating of the cold trap to 300 °C transferred the analytes through the transfer line (225 °C) to the GC equipped with a 30 m VF-5 ms column. The mass spectrometers were operated in electron ionization mode using full-scan (*m/z* 50-400).

Preliminary results

TSPE showed good chromatographic performance and minimization of co-eluting peaks. So far the method has showed to be quantitative within the range of VOC/SVOC from toluene to di-2-ethylhexyl phthalate (DEHP). The limit of detection of DEHP was estimated to 31 ng determined as three times the standard deviation of the mean of a low-concentration standard.

Whisky : For comparison several different whiskies brands (e.g. Ardbeg, Macallan and Glenlivet) were analyzed. The resulting chromatograms showed good chromatographic resolution and clear differences, thus facilitating (easy) distinction of the whiskies from one another. Phenols, cresols, acids and esters were identified as the main eluting compounds.

Floor dust : Comparison of floor dust samples from different locations in Denmark showed that the main constituents were phthalates (mainly DEHP), fatty acids and hydrocarbons. The main problem for direct on-column injection of floor dust extracts are the high content of fat. This problem was eliminated by TSPE.

Tattoo inks : A series of black tattoo inks (high content of carbon black pigment) were screened and compared for their content of organic compounds. They contained a large number of compounds including butanediol, phenol, trichlorobenzen, Texanol, fatty acid methyl esters, phthalates and PAHs.

The range of useful solvents is limited by the properties of Tenax TA to solvents like methanol, pentane and water. However, this may be extended to other solvents by use of other adsorbents.

TSPE has a large potential as a fast analytical method with minimal sample preparation for volatile and semi-volatile organic compounds in a wide range of sample matrices.

PTh-158
13:30 – 14:40

Organic coatings of engineered nanomaterials characterized by mass spectrometry

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Keywords:
Engineered nanomaterials, surface coating, GC-MS, LC-MS, NALDI

Novel aspects:
This is the first general approach using mass spectrometry for quantitative characterization of organic surface modifications of engineered nanomaterials.

Abstract:

Introduction

Engineered nanomaterials (ENM) are often chemically surface modified in order to tailor their physical-chemical properties for specific applications. For example, lipophilic surfaces are needed for incorporation of ENM in polymers whereas hydrophilic surfaces are used in water-based paints. These surface modifications may have influence on the toxicological and environmental properties of the ENM, but they are often trade secrets. Therefore, analytical procedures are needed to reliably enable detection and quantification of unknown ENM surface modifications. Only a limited number of publications describe quantitative methods for this type of application and despite of the obvious advantage of using mass spectrometry (MS) only a few studies describe the use of MS. Here we present an approach, based on several MS techniques, to characterize high temperature extractable and predominantly non-covalent bounded organic coatings of ENM.

Experimental

Thermogravimetric analysis (TGA) was used to identify ENM with organic coating. The following ENM with more than 1 wt % apparent coating were investigated: Organoclays, graphite, synthetic amorphous silica, titanium dioxide, silver, calcium carbonate, iron oxide, and nickel-zink-iron oxide. The ENM were extracted with pressurized liquid extraction (PLE) using methanol at 200 °C and 140 bar. The extract was centrifuged and the supernatant used for the succeeding analysis.

MS combined with on-column gas chromatography (GC-MS) or thermal desorption was used to analyze the extracts for volatile organic compounds. A 30 m 5 % phenylmethylsilicone column was used for GC and the MS was run in electron ionization (EI) mode and the mass range was m/z 50 to 500. The identification of organic compounds was based on searches in the NIST 2011 MS library and authentic standards.

Quadrupole time-of-flight MS combined with liquid chromatography (LC-MS) or direct infusion was used for analysis of non-volatile organic compounds in the extracts using both electrospray ionization and atmospheric pressure chemical ionization. The MS was operated in positive mode and the mass range was usually m/z 50 to 3000. Collision induced dissociation was used for structure determination.

Nanostructured surface-assisted laser desorption ionization time-of-flight MS (NALDI-TOF) was used for characterization of polymeric compounds. 1 µl of either a suspension of the ENM in methanol or the PLE extract was deposited on the NALDI target. Sodium trifluoro acetate was used as cationizing agent. Spectra were acquired in the reflector-positive mode and mass range was m/z 50-5000. The identification of the organic compounds from the NALDI-TOF results was based on pattern recognition, literature, and GC-MS data.

Results

A wide range of organic compounds was extractable from the ENM and their identity was confirmed by authentic standards in several cases. For example organoclays were surface functionalized with quaternary ammonium compounds containing benyzyl and alkyl side chains to make them miscible with polymers. Silver was surface modified by 2-pyrrolidone or coated with polyethoxylated nonionic surfactants. Graphite, calcium carbonates, iron oxides were coated with a mixture of fatty acids and fatty acid methyl esters - probably added to prevent agglomeration. Other compounds were silanes and siloxanes from synthetic amorphous silica and nickel-zink-iron oxide. Most of the extracted compounds are presumably intentionally added for different purposes. Some of the surface modifications have already been shown to influence the toxic properties of the ENM.

PTh-159
11:10 – 12:20

Simultaneous determination of cationic and anionic compounds using a high-speed polarity switching ESI and an online-SPE LC-MS/MS

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Keywords:
Online SPE LC-MS/MS, high-speed polarity switching, simultaneous determination, phthalate monoesters, phthalate diesters

Novel aspects:
Developed was simultaneous quantitation technique of cationic and anionic compounds which requires small volume of environmental samples and thus enables high through put screening of contaminants of emerging concern.

Abstract:
Mounting evidence suggesting ubiquitous presence of newly or unexpectedly detected chemicals, namely contaminants of emerging concern (CECs, e.g. pharmaceuticals and personal care products : PPCPs, perfluorinated compounds, steroid hormones and others) , has lead public concern and government actions. The CECs consist of hundreds of compounds, both cationic and anionic, and exist in the environment in generally trace levels. Those compounds typically enter the environment through sewage systems since the current wastewater treatment technologies are not designed for decomposing CECs, which is considered as an important exposure route for aquatic ecosystem. Some of the CECs are also found to resist drinking water treatment, which could result in human exposure. To establish an effective monitoring system for wastewater influent and drinking water, a high-throughput analytical method is in acute need.

For the analysis of trace level compounds, the current sampling methodologies often involve large volume sample collection to concentrate analytes in pretreatments, which limits sample transportation and introduces complex sample pretreatments. Recent advances in online solid phase extraction (online SPE) technology may be one of the solutions to this limitation since it requires relatively small volume of samples (typically 1-10 mL) and introduces all the injected volume into an analytical system. A newly developed high-speed polarity switching technology is capable of simultaneously determining cationic and anionic compounds including PPCPs, steroid hormones and other CECs.

An online SPE liquid chromatography tandem mass spectrometry (online SPE LC-MS/MS ; Shimadzu Corporation, Kyoto, Japan) was used to develop a simultaneous quantitation method for the analysis of phthalate di- and mono-esters (PEs) in finished water. One mL of raw samples was injected after receiving stable isotope labelled PEs. Two binary pumps were used for sample cleaning with an SPE column (MASK-ENV, Chemco Scientific Co., Osaka, Japan) and another two pumps were used for separation by an analytical column (Shim-pack XR-ODS) . For both sets of pumps, scrubber columns (Shim-pack XR-ODS) were inserted just after mixing chambers to reduce contaminations from the analytical system.

Method detection limits (MDLs in ng/ml) were as follows: monometyl- (MMP, 0.203) , monoetyl (MEP, 0.162) , mono-*n*-butyl (MnBP, 0.214) , monobenzyl (MBzP, 0.201) , mono-2-ethylhexyl- (MEHP, 0.213) , mono-2-ethyl-5-hydroxyhexyl- (MEHHP, 0.223) phthalates; dimethyl- (DMP, 0.144) , dietyl- (DEP, 0.179) , di-*n*-butyl- (DnBP, 1.43) , butylbenzyl- (BBzP, 0.117) , di- (2-etylhexyl) - (DEHP, not calculated due to blank contamination) phthalates. Recoveries ranged 56 %-134 %. DnBP and DEHP were detected even in procedure blanks suggesting that contaminations occurred during sample preparations or analytical procedures. Phthalate monoesters were not detected or below MDLs in the most of samples although MEHP was detected in one sample (0.471 ppb) . Since this method is capable to detect very low levels of PEs, it is essential to minimize contaminations from sampling to analysis in order to achieve lower MDLs for phthalate diesters. In the presentation, preliminary result of the application of online-SPE-LC-MS/MS system for the measurement of phthalate esters will be discussed.

PTh-160
13:30 – 14:40

The analysis of olefine and aromatic hydrocarbon in hydrocarbon mixture using Multi-Dimensional GC/GCMS system

LiBin Liu, GuiXiang Yang, TaoHong Huang, Lei Cao

Shimadzu Co.,Beijing, China

Keywords:
hydrocarbon mixture,Multi-Dimensional GC/GCMS system

Novel aspects:
MDGC/GCMS system can show superiority on the analysis of complicated matrix sample such as hydrocarbon mixture,etc.

Abstract:
The component of hydrocarbon mixture is very complicated, including even and odd number carbon hydrocarbon. Its analysis with conventional gas chromatographic approaches is a big challenge and can give us inaccurate or even false results because overlapping is always happened. Many measures were taken to avoid interferences, such as improving sample preparation or using high selectivity detectors. Multi-dimensional GC/GCMS system with multiple heart-cutting is one of the powerful tools. Multi-dimensional GC/GCMS can improve resolution beyond that of the regular GC analysis as it re-introduces the dissolved component of interest into another column. In other words, only part of the peak of the component that was insufficiently separated on the column where the sample initially passed through (called the "1st column") is introduced (heart-cut) to a column of another type (called the "2nd column"), so that insufficiently separated components can be separated. A device called a "switching device" is used for heart-cut introduction of peaks eluted from the 1st column to the 2nd column. As a switching device, the recently developed Multi-Deans switching unit can be used in combination with a GC-FID as the first analytical dimension and a GCMS as the second analytical dimension. The analytes pass the first column and are detected in the FID ("stand-by mode") or are transferred to the second column and analyzed with mass spectrometer or GC detector such as FID ("cut mode"). By using this system, complicated matrix analysis such as hydrocarbon mixture was done to demonstrate MDGC/GCMS system performance.
This MDGC/GCMS system can analyze the olefine and aromatic hydrocarbon in hydrocarbon mixture. It has superiority on the determination of the complicated matrix sample in order to obtain more reliable analytical results.

PTh-161
11:10 – 12:20

A Novel Sensing Material for Iron(III) Ions based on Poly (gamma-Glutamic acid)-grafted-3,4-dihydro-3-2'-ethylhydroxyl-6-methyl-1,3,2H-benzoxazine

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Keywords:
Poly (gamma-glutamic acid) , benzoxazine, Fe (III) ion, colorimetric sensing material, photometric titration method

Novel aspects:
The novel polymeric sensing materials was synthesized based on a simple reaction. The responsiveness of the polymer toward Fe (III) is very rapid, and clearly observable with the naked eye.

Abstract:
A novel sensing material for Fe (III) ion was prepared from poly (gamma-glutamic acid) (gamma-PGA) and 3,4-dihydro-3-(2'-ethylhydroxyl)-6-methyl-1,3,2H-benzoxazine (Mt-Bx) . Mt-Bx was used as an ionophore segment and grafted onto the gamma-PGA backbone via an esterification reaction. The optimum reaction time determined by FT-IR was 2 h. The calculation based on ^{1}H-NMR spectrum revealed that the most attainable grafting degree was 30%. This copolymer showed a highly selective and sensitive recognition toward Fe (III) ions. A simultaneous transition of color and solubility was observed when the copolymer formed complex with the Fe (III) ions. These responses were clearly observable with the naked eye. A quantitative analysis based on a photometric titration method indicated that the copolymer exhibited and excellent interaction with Fe (III) ions at a stoichiometric ratio of 1 : 390.

PTh-162
13:30 – 14:40

Analysis of perfluorinated compounds in sediment samples from wastewater canal of Pancevo industrial area, Serbia

Vladimir P Beskoski[1], Shuusuke Takemine[2], Takeshi Nakano[3], Latinka Slavkovic-Beskoski[4], Gordana Gojgic-Cvijovic[1], Mila Ilic[1], Srdjan Miletic[1], Miroslav M Vrvic[1,5]

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Keywords:
PFOA, PFOS, Industrial wastewater canal, Sediment, Danube River

Novel aspects:
This is the first report of presence of PFCs compounds in the sediments from Serbia. Compared to other reports, high levels of PFOA and PFOS were found.

Abstract:
Perfluorinated compounds (PFCs) are chemicals that do not occur naturally, but have been widely used in chemical production for some time. They are globally distributed, environmentally persistent, bioaccumulative, and potentially harmful. Perfluorooctansulfonate (PFOS) and perfluorooctanoate (PFOA) are the two PFCs most commonly used and found in the environment. Together with perfluorohexane sulfonate (PFHxS) these compounds are widely employed in different industrial processes such as in protective coatings.
The wastewater canal (WWC) Vojlovica was built in 1962 to collect the wastewater discharges from the industrial complex of the city of Pancevo in Serbia. Industrial complex consist of a petrochemical factory (HIP Petrohemija) , an oil refinery (NIS Rafinerija, Pancevo) and chemical fertilizers factory (HIP Azotara) . The canal is artificial with no natural flows, about 2 km long, around 70 m wide and directly connected to the Danube River. The water depth is around 12 m. The environment surrounding the canal has been strongly affected for a long time by the presence of the industrial complex. Additionally heavy destruction during NATO bombing events in 1999 resulted in contamination of air, soil, groundwater and the WWC itself.
In total, 4 sediment samples from WWC were collected. Surface sediments layer of 15 cm were taken by a Van Veen Grab sampler, transported in glass jars and stored in the laboratory at 4 °C. For comparative purposes, the same type of sample were also taken from the navigation canal flowing parallel to WWC but not receiving any direct discharge of industrial wastewaters.
Sampling sites are listed below :
No 1 - navigation canal ;
No 2 - at the confluence of WWC with the Danube River, downstream from the industrial area and effluents ;
No 3 - downstream from the fertilizer factory outlet (first effluent) ;
No 4 - downstream from the petrochemical plant (second effluent) ;
No 5 - downstream from the oil refinery outlet (third effluent) .
Sediment sample was extracted with methanol. MPFAC-MXA as mass-labeled surrogates was spiked into the sample. The sample was extracted with SPE. The elution was concentrated and labeled $^{13}C_8$PFOA was added as syringe spike. The each final solution was analyzed by liquid chromatography (LC) -tandem mass spectrometer (MS/MS) using Xevo TQ (Waters) coupled with ACQUITY UPLC (Waters) .
Concentrations of PFCs were determined as follows :
No 1 : 68, 230 and 230 ng/kg-dry of PFOA, PFHxS and PFOS, respectively.
No 2 : 80 and 2100 ng/kg-dry of PFOA and PFOS, respectively.
No 3 : 170 and 5300 ng/kg-dry of PFHxS and PFOS, respectively.
No 4 : 130, 170, and 5700 ng/kg-dry of PFOA, PFHxA, and PFOS, respectively.
No 5 : 76, 66 and 420 ng/kg-dry of PFOA, PFHxA, and PFOS, respectively.
Concentrations of PFOS in the samples No 3 and No 4 are 3 -3.2 times higher compared with sea sediment in Tokyo bay[1)]. PFOA and PFOS concentrations from WWC were from two to twenty fold higher comparing to sediment samples taken from Roter Main river (Germany) which receives treated waste waters of industrial, commercial and domestic origin from municipal wastewater treatment plant[2)]. Comparing to upstream Danube River bank sediment samples[3)] PFOS from the WWC samples were from two to six fold higher.
This is the first study and report of presence of PFCs compounds in the samples from Serbia. Most of the PFCs are released from fertilizer factory and petrochemical plant outlets, while oil refinery outlet mostly contribute to petroleum pollution. The exact origin of PFCs cannot be established from one study but one of the reasons for presence of these compounds might be their usage as components in pipes, fittings and wiring insulations.

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3) Clara, M.et al *Water Research* 43, 4760-4768 (2010)

PTh-163
11:10 – 12:20

Study of On-site sampling method for Dioxin in water with high concentrations of suspended solids.

Takeshi Enomoto[1], Miho Okimoto[1], Chuanpit Boonyoy[2], Areerat Jaksakul[2], Ruchaya Boonyatumanond[2], Genta Takahashi[5], Kenji Tawara[5], Tohru Matsumura[3], Takeshi Nakano[4]

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Keywords:
Dioxin, PCB, river water, coagulant, Suspended Solids

Novel aspects:
We have been developed the water sampling method by applyingcoagulant to analysis of dioxin in water which has a large volume of suspended solids.

Abstract:
Dioxins in water are generally collected by disk type SPE with ODS. However, a large amount of water causes clogging of the disk and delays time of the sampling. In order to resolve this problem, adsorbents utilizing coagulation have been developed. We have been developed the water sampling method by applying coagulant to analysis of dioxin in water which has a large volume of suspended solids. SS can result from erosion from urban runoff and agricultural land, industrial wastes. Suspended solids (SS) are solid materials, including organic and inorganic, that are suspended in the water. SS consists of silt, plankton and industrial wastes. Samples are stably collected in spiking experiments using river water. Because this method requires minimum instruments and takes shorter time to analysis, it has more advantage than the conventional water sampling method.
In this study, congener-specific analysis of PCDD/DF including PCBs were performed in the environmental samples using BPX-Dioxin, HT 8 -PCB as separation column by GC/HRMS (JEOL JMS-800D) . HT 8 -PCB capillary column (60m, 0.25mm i.d.) was used for determination of PCB all congeners. Contribution of PCB-11 in the environmental samples such as river water and ambient air were evaluated.
PCB congener profiles in environmental samples, such as air, water, and sediment, are similar to the pattern of a particular PCB product used around the area. On the other hand, among biological samples, such as fish, clams, birds and humans, the congener profiles differ drastically reflecting different half-lives of congeners in different biological systems. We would propose the source estimation and monitoring method using PCB congener-specific analysis.

PTh-164
13:30 – 14:40

Multi-residue analysis of pesticides in animal and fishery products, and their processed foods by dual-column GC-MS/MS

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Keywords:
pesticide, animal and fishery product, processed food, dual-column, GC-MS/MS

Novel aspects:
Newly multi-residue method to enable the analysis of water-soluble pesticides such as methamidophos in processed foods such as frozen gyoza dumplings, and highly quantification at ppb levels by dual-column GC-MS/MS.

Abstract:
In Japan, food poisoning caused by methamidophos-tainted frozen gyoza dumplings imported from China came to light in January 2008. The news has set off alarms about the safety of not only perishable foods but also processed foods. Methamidophos is a water-soluble pesticide (solubility in water >200 g/L and Kow logP =-0.8 at 20&#8451 ;) . The official multi-residue method for animal and fishery products is only applied to fat-soluble compounds (Kow logP > 2 - 3) such as DDT's in perishable foods. Therefore, we developed a multi-residue method for determining many pesticides including water-soluble compounds (Kow logP >- 1) in animal and fishery products, and their processed foods by dual-column gas chromatography with tandem quadrupole mass spectrometry (dual-column GC-MS/MS) .

First, 21 stable isotopically labeled pesticides, dichlorvos-d_6, acephate-d_6, α-HCH-d_6, diazinon-d_{10}, iprobenfos-d_7, carbaryl-d_7, fenitrothion-d_6, linuron-d_6, metolachlor-d_6, chlorpyrifos-d_{10}, diethofencarb-d_7, fosthiazate-d_5, pendimethalin-d_5, thiabendazole-$^{13}C_6$, imazalil-d_5, isoprothiolane-d_4, isoxathion-d_{10}, *pp'*-DDT-d_8, EPN-d_5, etofenprox-d_5, es-fenvalerate-d_7 and triphenylphosphate as the internal standards (surrogates) for 202 representative target pesticides were selected. The chopped sample spiked with surrogates was homogenized with 2 % acetic acid aqueous solution, and extracted with acetone-*n*-hexane (2 : 3) while melting adipose tissue under mildly acidic conditions, and then centrifuged. The organic layer and aqueous layer were filtered together. The remaining solid contents were re-extracted with acetone-*n*-hexane (2 : 3) , and then filtered. After concentration of the combined filtrate, the aqueous residues saturated with sodium chloride was loaded onto a macroporous diatomaceous earth column (EXtrelut NT20) , and eluted with ethyl acetate. Co-extractives were removed by a combination of gel permeation chromatography (GPC, CLNpak EV-2000AC-12F column : 300 x 12 mm id, CLNpak EV-G-12F guard column : 100 x 12 mm id, mobile phase : acetone-cyclohexane (3 : 17)) and graphitized carbon solid-phase extraction (SPE, Carbo C-Avicel (1 : 4) : 500 mg, eluant : acetone-cyclohexane (3 : 17)) automatically, and then by a tandem SAX/PSA cartridge SPE (Bond Elut Jr-SAX : 500 mg, Bond Elut Jr-PSA : 500 mg, eluant : acetone-*n*-hexane (1 : 1)) . The cleaned sample extract was subjected to dual-column GC-MS/MS (Rxi- 5 Sil MS first column : 30 m x 0.25 mm id, Rtx-200MS secondary column : 30 m x 0.25 mm id, ionization mode : EI, MS/MS mode : MRM) .

Mean recoveries (n = 5) of most pesticides from ten kinds of fortified samples, beef, salmon, hen's egg, cow's milk, honey, kimchi, corned beef, eel kabayaki, frozen gyoza dumpling and retort curry at 0.01 mg/kg were within a range from 70 to 120 % (relative standard deviation values <15%) .

The method was applied to many commercial foods to demonstrate its use in routine analysis. Not a few interfering peaks appeared on the MRM chromatograms of some food such as retort curry at ppb level detection. In these cases, the most of interfering peaks did not disappear by using alternative MRM transitions, so that using a single-column GC-MS/MS may result in false positive data. To overcome this problem, GC-MS/MS was equipped with a secondary column together. As a result, no interfering peaks on the either MRM chromatograms of almost all foods were detected. The capability of the best column combination for GC-MS/MS allows easy identification and precise quantification of many pesticides in animal and fishery products, and their processed foods at the ppb level such as uniform limit (0.01 mg/kg) under the Japanese positive list system.

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PTh-165
11:10 – 12:20

Simultaneous analysis of cationic, anionic and neutral surfactants from different matrices using LCMS/MS.

Rashi Kochhar[1], Shruti Raju[1], Deepti Bhandarkar[1], Bhairavi Saraf[1], Shailendra Rane[1], Jitendra Kelkar[1], Ajit Datar[1], Zhaoqi Zhan[2]

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Keywords:
Surfactants, Ultra fast MS, Polarity switiching, Triple Quadrupole, Industrial Waste ,

Novel aspects:
LCMS/MS with fast scanning and polarity switching is a good screening technique for analysis of surfactants in areas like pharmaceuticals, forensics, effluent treatments, cosmetics etc.

Abstract:
Surfactants are compounds that lower the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. They are routinely deposited in numerous ways on land and into water systems, whether as part of an intended process or as industrial and household waste. Some surfactants are known to be toxic to animals, ecosystems, and humans, and can increase the diffusion of other environmental contaminants. Some detergents also cause lysis of cells and tissues by disorganizing the cell membrane's lipidic bilayer and solubilising proteins.
It, therefore, becomes important to detect these surfactants and here we have attempted to simultaneously analyse them
The surfactant standards were dissolved in water : methanol (1 : 1) to ensure all surfactants can be solubilized, and subjected to liquid chromatography using UHPLC Nexera coupled with triple quadrupole (TQ) mass spectrometer LCMS 8030, Shimadzu Corporation. The samples were subjected to Solid Phase Extraction (SPE) and analysed using the method developed for the standards. The chromatographic condition ensured analysis to be completed in short time, because of system tolerance to high pressure. Subsequent fast analysis was supported by Fast TQ MS.
The surfactant standard mixture was separated chromatographically and subjected to mass spectrometry. Surfactants of different ionising tendencies were simultaneously analysed for their parent and product ions. For eg, Cetrimide, Octylphenol ethoxylate were analysed in ESI positive mode, whereas Sodium lauryl sulphate (SLS) was analysed in ESI negative mode. Molecular ion of cetrimide was observed with loss of bromine whereas molecular ion of SLS was observed with loss of sodium. Octylphenol ethoxylate showed a characteristic polymeric pattern with mass difference of 44. Fast analysis was possible due to short runtimes that could be achieved without compromising resolution in Nexera system. The ultrafast polarity switching and ultrafast scanning capacity of the mass spectrometric system enabled analysis of different types of molecules in the same run.

PTh-166

13:30 – 14:40

Detection of Melamine in Human Renal Uric Acid Stone by Matrix-Assisted Laser Desorption / Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

Chia-Fang Wu[1], Chia-Chu Liu[2,3,4,5], Jentaie Shiea[6], Yi-Tzu Cho[7], Yii-Her Chou[2,3], Bai-Hsiun Chen[8], Chao-Yi Chien[1], Shu-Pin Huang[2,3], Wen-Jeng Wu[2,3,9], Jung-Tsung Shen[9], Mei-Yu Chang[9], Chun-Hsiung Huang[2,3], Ai-Wen Chang[2], Ming-Tsang Wu*[1,4,10,11]

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Keywords:
melamine, urolithiasis, MALDI-TOF MS

Novel aspects:
MALDI-TOF MS could detect melamine in uric acid stone specimens, even when urinary melamine level was undetectable, which can be an additional analytical method to evaluate for external melamine exposure.

Abstract:
Background : The link between melamine-contaminated daily foodstuffs and urolithiasis formation has drawn international concern. However, detection of melamine levels in urine may not completely represent external melamine exposure. Thus, finding an additional analytical method for the study of environmental melamine exposure and its adverse effect in humans is crucial.
Methods : Eleven adult patients diagnosed with uric acid urolithiasis were retrospectively analyzed. Melamine levels in their overnight one-spot urine samples were measured by a triple quadrupole liquid chromatography tandem mass spectrometry (LC-MS/MS) . The compositions of stone samples were analyzed by the Fourier transform infrared (FTIR) spectrophotometer and matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF MS) .
Results : Seven (63.6%) out of 11 patients had detectable melamine levels in their urine specimens (method of detection limit : 0.8 ng/ml) . Three patients (27.3%) were highly suspected of having melamine-containing urolithiasis in FTIR spectra. In one of those three cases who still had available stored stone specimens, MALDI-TOF MS further confirmed melamine components in this male patient's stone specimens. In contrast, his urinary melamine level was below the detection limit by LC-MS/MS.
Conclusions : Direct analysis of melamine in the composition of urolithiasis by MALDI-TOF MS can be an additional analytical method to evaluate for external melamine exposure.

*Correspondence : Prof Ming-Tsang Wu

PTh-167
11:10 – 12:20

Urinary oxidative metabolites of di(2-ethylhexyl)phthalate can predict the daily intake of phthalate-tainted foods in Taiwanese children

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Keywords:
urinary phthalate metabolites, children, LC-MS/MS

Novel aspects:
Urinary oxidative metabolites of DEHP of our study children can predit their affected foods exposure.

Abstract:
Background : A major incident of phthalate-contaminated foodstuffs happened in Taiwan between April and July, 2011. Phthalates, mainly di (2-ethylhexyl) phthalate (DEHP), were deliberately added to foodstuffs as a substitute of emulsifier. The highest concentration of DEHP in affected foods belonged to the category of nutrient supplements which were regularly taken by children or infant. Thus, this study investigated the correlation between the amount of DEHP intake and its major oxidative metabolites in Taiwanese children.

Methods : Between May 31 and June 17, 2011, we established a special Phthalates Clinic for Children (PCC) at Kaohsiung Medical University Hospital in southern Taiwan for those children who were suspected to have the intake of affected foods. Their parents were interviewed to collect their exposure information from two sources : the official website of Taiwan Food and Drug Administration and Bureau of Health of Kaohsiung City to calculate daily intake dose. One-spot urine specimen in each study children was collected and measured four oxidative metabolites of DEHP, including 5 oxo-MEHP, 5 OH-MEHP, 5 carboxy-MEPP, and 2 carboxy-MMHP, by a triple quadrupole liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results : In total, 28 children whose age £ 10 years old (pre-puberty) were studied. The daily intake dose ranged from 0 to 0.16 mg/kg/day. The intake of affected foods in 19 (67.9%) and 12 (42.9%) out of 28 study children exceeded the tolerable daily intake (TDI) recommended by U.S. Environmental Protection Agency (TDI < 0.02 mg/kg/day) and European Food Safety Authority (TDI < 0.05 mg/kg/day). For urinary DEHP metabolites, after corrected by urinary creatinine (Cr), the median levels (interquartile range) of 5 oxo-MEHP, 5 OH-MEHP, 5 carboxy-MEPP, and 2 carboxy-MMHP were 76.09, 80.36, 109.31, and 36.84 μg/g Cr (56.21-143.32, 29.28-137.36, 45.98-200.33, and 24.74-59.40 μg/g Cr, respectively). We found that the significant and positive correlations between DEHP daily intake (mg/kg/day) and urinary 5 oxo-MEHP (Spearman correlation r=0.39, p=0.04) and 5 carboxy-MEPP (r=0.45, p=0.02), but not for 5 OH-MEHP (r=0.28, p=0.14) and 2 carboxy-MMHP (r=0.32, p=0.10).

Conclusions : These study children were exposed to the high amount of DEHP from affected foods and some oxidative metabolites of DEHP was correlated to their exposure dose. A further study is necessary to link these exposure indices to the health outcome in children.

*Correspondence : Prof Ming-Tsang Wu

PTh-168
13:30 – 14:40

LC-MS analysis of neonicotinoid insecticides in the crops using a novel selective solid-phase extractant having dipole type functional group

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Keywords:
SPE, Adsorbent, LC-MS, Neonicotinoids

Novel aspects:
We have developed a novel adsorbent which highly recognized chemical characteristics of the functional groups.

Abstract:
Neonicotinoids, which are insecticides chemically related to nicotine, act as an agonist on an acetylcholine receptor in insects, as nicotine does. Since neonicotinoids show much lower toxicity in mammals, 7 kinds of them represented by imidacloprid are currently the most widely used insecticide in the world. Chromatographic methods dedicated to the determination of insecticide residues such as GC and HPLC have been reported. However, they require a fair amount of sample manipulations such as liquid-liquid extraction and column cleanup procedure, and do not fit for a distribution cycle of food.

The basic chemical structure of the neonicotinoids contains both heteroaromatic ring and dipole functional group such as nitroimine or cyanoimine. We have developed a novel adsorbent, PNP, which highly recognized chemical characteristics of the functional groups. The PNP adsorbent was synthesized by introducing *p*-nitrophenol on the hydrophilic base gel with glycidyl group. One hundred mg dry weight of the adsorbent was packed in a solid-phase extraction cartridge. The cartridge was conditioned by passing 10 mL of acetone, and then neonicotinoids mix solution dissolved in various solvents were loaded on it. The solutes concentration in various eluates from the cartridge was measured by LC-MS.

Neonicotinoids in acetone or ethyl acetate were tightly entrapped on the PNP adsorbent and were easily eluted with methanol. It appeared that development of plural interaction such as hydrogen bonding, π-π interaction, and dipole interaction raised selectivity of the adsorbent for neonicotinoids. Acetone or ethyl acetate is suitable extraction solvent for pesticides of wide polarity ranges. Thus, the PNP adsorbent was used to determine neonicotinoids in the actual crops such as green pepper. Quantitative analysis was done in selected-ion monitoring mode for each compound. The use of PNP adsorbent allows for clean extracts to be obtained which dramatically reduces sample matrices.

PTh-169 Studies on Residual Characteristics of Growth Regulator 6-BA in Bean Sprout

11:10 – 12:20

Wan-Hee Seo, Young-Mo Jeong, Soon-Kil Cho, Bong-Suk Oh

Jeonnam Provincial Office, National Agricultural Products Quality Management Service, MIFAFF, Korea

Keywords:
Bean sprout, Residue analysis, LC-MS/MS

Novel aspects:
We think 6-BA will not be remained during its shipping date.

Abstract:
Bean sprout is a common food due to its cheap price and short cultivation period (5-7 days). It is also used as an ingredient of in-flight meal and food for astronauts. Industrial mass production of bean sprout usually accompanied diverse chemical drugs such as seed disinfectants and growth regulators. In this study, residual characteristics of growth regulator 6-BA were observed. During the cultivation of bean sprout, the seeds were washed and soaked in water for 20 hours. 6-BA was applied (75 mL/6 L/75 kg) to the seeds and 1.2 kg bean sprout seeds were cultivated in plastic container for 7 days with sprinkling of water (15 min/4 h). Temperature and humidity were maintained as 18.3±0.6℃ and 71.0±1.6 % during the cultivation period. After 7 days, the weight of bean sprout was increased from 1.6 g to 14 g. For sample preparation, each sample was extracted with 30 mL acetonitrile and kept in a refrigerator (-80℃) for 5 min in order to separate organic and aqueous layer. The organic supernatant was directly injected to LC-MS/MS for residual analysis of 6-BA. The method was validated in triplicate at two different fortification concentrations (0.1and 0.5 mg/kg) in the blank matrix. Good recoveries were observed for the target analysis and ranged between 78.2 and 84.9% with relative standard deviations of less than 10%. The limits of detection (LOD) and limits of quantification (LOQ) were 0.003 and 0.01 mg/kg for 6-BA. The residual amount of 6-BA in application day was 1.60 mg/kg. A slow degradation of 6-BA was observed until 5 days of application with a concentration of 0.07 mg/kg. After 6 days, 6-BA was completely disappeared in the bean sprout samples. Based on the dissipation pattern, we think 6-BA will not be remained during its shipping date.

PTh-170
13:30 – 14:40

Improvement of Determination Method for Pesticide Residues in Bean Sprout

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Keywords:
Improved method , LC-MS/MS

Novel aspects:
The improved method is easy and reliable for the determination of carbendazim, thiabendazole, and 6-BA in bean sprout.

Abstract:
Bean sprout is a common food due to its short cultivation period and cheap price. An industrial mass production accompanied diverse chemical drugs, such as seed disinfectants and growth regulators, which led to controversies regarding its safety. The treatment of carbendazim and thiabendazole to bean sprout was legally allowed, but their residues have to be absent according to their maximum residue limits in bean sprout. Typical methods used for the determination of the pesticide residues in bean sprout have tended to be labor-intensive and have used large volumes of solvent, resulting in large volumes of hazardous waste. It is also very difficult to monitoring pesticides in bean sprout due to its short distribution period. Thus, the typical sample preparation needs to minimize the number of steps in order to reduce both time and sources of error during theses process. For sample preparation in this study, precisely 20 grams of samples were placed into a centrifuge tube. A 20 mL portion of acetonitrile was added, and vigorously shaken for 5 min followed by centrifugation at 3000 rpm for 5 min. The tube was kept in a refrigerator (-80&#8451 ;) for 5 ~ 10 minute to separate acetonitrile and water layers. A 2 mL portion of the acetonitrile layer was injected into a LC-MS/MS. Recoveries were between 77.1 and 98.7 for carbendazim, thiabendazole, and 6-BA. The calculated LOQs were 0.01 mg/kg for 6-BA, and 0.001 mg/kg for carbendazim and thiabendazole. These results indicate that the improved method is easy and reliable for the determination of carbendazim, thiabendazole, and 6-BA in bean sprout.

PTh-171
11:10 – 12:20

Screening of five mycotoxins by using immunoaffinity column and HPLC-orbitrapMS in processed foods

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Keywords:

Mycotoxins, OrbitrapMS, Immunoaffinity column

Novel aspects:

Applying to processing foods, Using OrbitrapMS, Muti-determination of mycotoxins

Abstract:

Introduction

The risk assessment of mycotoxins contamination of foods was important because it can cause harmful health effects (e.g. cancer) in the humans. But the determination of mycotoxins in processing foods (e.g. snack, instant noodle) was a difficult experiment due to residue of extremely small amounts and adverse matrix effects (e.g. fat and sugar) . The object of this study was to development a reliable and fast analytical method using by immunoaffinity column and HPLC-Orbitrap MS system in processing foods.

-Five mycotoxins : Aflatoxin, DON, Zearalenone, Ochratoxin, Fumonisin

Materials & Methods

-Sample preparation : The immunoaffinity column was applied to ensure efficient cleanup. The homogenized 10g sample was weighed. 50mL of PBS (1 X, pH 7.4, 1 st extraction) and 50mL of 70% MeOH in DW (2 nd extraction) were added for extraction. Two aliquot of the extract were transferred into immunoaffinity column for cleanup. After drying down eluant under an nitrogen stream at 50&#8451 ; , reconstitute with 1 mL of 40% MeOH in DW containing 1 mM ammonium acetate and 0.1% acetic acid.

-LC/MS/MS analysis : 10uL of preparation sample was injected onto a XBridge C18 150*2.1mm, 3.5uL analytical column. A gradient LC method used mobile phases water containing 1 mM ammonium acetate and 0.1% acetic acid and methanol containing 1 mM ammonium acetate and 0.1% acetic acid at a flow rate of 0.2mL/min. HPLC-Orbitrap MS with ESI (+,-) probe was used and scan type was full scan.

Data & Results

- MS Condition : Aflatoxin B 1 (313.0707,+) , Aflatoxin B 2 (315.0863,+) , Aflatoxin G 1 (329.0656,+) , Aflatoxin G 2 (331.0812,+) , Fumonisin B 1 (722.3957,+) , Fumonisin B 2 (706.4008,+) , DON (355.1387,+) , Zearalenone (317.1394,-) , Ochratoxin (404.0901,+)
- Linearity : The calibration curves had R^ 2 values that were greater than 0.98.
- Recovery : Each test was performed three times and two spiked level. In snacks, the mean recovery values range from 79.0% to 116.7% and in instant noodles from 73.8% to 94.7%.
- Repeatability : The RSD was calculated from three replicates. In snacks, the RSD range from 10.6% to 17.9% and in instant noodles from 8.5% to 14.4%.
- LOQ : The level of LOQ was low than the MRL established KFDA (Korea Food and Drug Administration) for mycotoxins. LOQ range of aflatoxin, DON, zearalenone, ochratoxin, and fumonisin were 0.1, 10.0, 5.0, 0.5, and 10.0ug/kg respectively.

PTh-172
13:30 – 14:40

Simultaneous determination of melamine and its analogues in various processed foods using LTQ-orbitrap HRMS

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Keywords:
Melamin, LC-MS/MS, LTQ-orbitrap HRMS, GC-MS

Novel aspects:
Simultaneous analysis of melamine, ammeline, ammelide and cyanuric acid in various processed foods by LTQ-orbitrap HRMS is presented.

Abstract:
[Introduction]
Various techniques have been used to detect melamine and cyanuric acid. But limited methods including GC-MS are suitable for the simultaneous determination of melamine, ammeline, ammelide and cyanuric acid.
However, derivatization is necessary to GC-MS detection, and comparatively poor reproducibility of the quantitative analysis may be unavoidable due to the incomplete derivatization.
At the same time, because of the large polarity of melamine and its related compounds, RP-HPLC method has weak retention and poor separation efficiency.
The purpose of our study was to develop a LTQ-orbitrap HRMS method for the simultaneous determination of melamine and its analogues in processed foods (e.g. instant noodle soups and milk products) .

[Methods]
The US FDA method for melamine and cyanuric acid was followed with some modifications made to establish a method for all four compounds.
The sample was separated on a Biobasic AX column with acetonitrile-10 mM ammonium acetate (78 : 22, v/v) as the mobile phase at flow rate 0.2 mL/min, and determined by positive and negative ESI mode. Test sample was extracted with 2.5% formic acid. The extract was centrifuged and the supernatant was determined by LC-MS/MS and confirmed by LTQ-orbitrap HRMS. Separation was achieved within 10 minutes using a column.
SPE cartridge was used as a clean-up filter to retain matrix interferences while all the polar analytes pass through.
($^{15}N_3$,$^{13}C_3$) -melamine, ($^{15}N_3$,$^{13}C_3$) -cyanuric acid, $^{13}C_3$-ammeline and $^{13}C_3$-ammelide were used as internal standard, respectively.

[Preliminary data]
Under the optimized conditions, the retention times of melamine, ammeline, cyanuric acid and ammelide were about 2.8, 3.6, 4.0 and 7.2min, respectively.
For each analyte, a single mass spectral peak corresponding to the [M+H] + ion or [M-H] - ion was observed. Accordingly, the [M+H] + ions were used as precursor ions for melamine and ammeline, while the [M-H] - ions were used as precursor ions for ammelide and cyanuric acid.
Accuracy mass : melamine (+, $C_3H_6O_6$, 127.07267) , cyanuric acid (-, $C_3H_3N_3O_3$, 128.00907) , ammeline (+, $C_3H_5N_5O$, 128.05669) and ammelide (+, $C_4H_4N_4O_2$, 129.04070)
Isotope accuracy mass : melamine (+, $^{13}C_3$, $^{15}N_3$, 133.07384) , cyanuric acid (-, $^{13}C_3$, $^{15}N_3$, 134.01024) , ammeline (+, $^{13}C_3$, 131.06675) and ammelide (+, $^{13}C_3$, 132.05076)
The limit of detection was 0.01 mg/kg and the limit of quantification was 0.5 mg/kg. The RSDs of the retention times and peak areas were 0.02~0.80 and 1.01~2.80%, respectively. The mean recoveries were varied from 85.0 to 102.1% for four targets in the various instant noodle soups and milk products.
The method was validated and good results were obtained with respect to precision, repeatability and spiked recovery,

PTh-174
13:30 – 14:40

Determination of DNA adducts originating from methyleugenol using isotope-dilution UPLC-ESI-MS/MS

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Keywords:
DNA adducts, UPLC-ESI-MS/MS, methyleugenol, MRM quantification

Novel aspects:
Development and application of UPLC-ESI-MS/MS method for quantification of DNA adducts originated from methyleugenol

Abstract:
Methyleugenol (ME) is a secondary metabolite present in many herbal spices. Former observations indicate that hydroxylation followed by sulfation is an important bioactivation pathway of ME and other alkenylbenzenes leading to carcinogenicity in animals. In this context, DNA adducts of activated alkenylbenzenes have been detected *in vitro* and *in vivo* using the ^{32}P-postlabeling assay. This method cannot provide structural information of the detected adducts. Therefore, the aim of the present study was to characterize the chemical structures of ME-derived DNA adducts on the basis of MS-MS fragmentation patterns. Furthermore, we developed a sensitive quantification method using UPLC-ESI-MS/MS suitable for studying the bioactivation as well as adduct formation potential of ME *in vitro* and in animal models.
The UPLC-ESI-MS/MS method developed is based on the analytical determination of adducted 2'-deoxynucleosides using isotopic dilution analysis. For this purpose, stable-isotope labeled adduct standards of the ME-derived 2'-deoxyguanosine adduct and 2'-deoxyadenosine adduct were synthesized.
Extracted DNA is enzymatically hydrolyzed into 2'-deoxynucleosides with addition of stable-isotope labeled compounds as internal standards. Afterwards the analytes are separated from digestion mixture ingredients by protein precipitation using ethanol. The 2'-deoxynucleoside adducts are chromatographically separated within 4 minutes. MRM data are aquired in the positive ion mode using three transitions for each adduct. All analyses are conducted with an ACQUITY UPLC (Waters) connected to a Xevo™ TQ MS (Waters).
By means of daughter scan experiments we were able to identify the structures of DNA adducts originated from ME, which were confirmed by NMR data. Consequently we generated MRM methods for these adducts. Therefore, we could identify and absolutely quantify adducted 2'-deoxyguanosine and 2'-deoxyadenosine in samples resulting from *in vitro* and *in vivo* experiments. The achieved sensitivity of this method is nearly one adduct per 10^8 nucleosides.
In summary the new developed method represents a suitable and improved alternative for detection of ME-derived DNA adducts. Furthermore,ME-derived RNA adducts can be analyzed in the same UPLC run applying RNA adduct specific MS-MS transitions.
By means of this method we investigated the bioactivation pathway of ME via sulfation as well as the DNA adduct formation potential *in vitro* (1) and *in vivo*. Moreover, we even could demonstrate the presence of ME-derived DNA adducts in human tissues.

(1) Herrmann, K., Engst, W., Appel, K. E., Monien, B. H., Glatt, H. (2012) Identification of human and murine sulfotransferases able to activate hydroxylated metabolites of methyleugenol to mutagens in *Salmonella typhimurium* and detection of associated DNA adducts using UPLC-MS/MS methods. *Mutagenesis*, (in press) doi : 10.1093/mutage/ges004.

PTh-175
11:10 – 12:20

Exploring the application of a universal method for pesticide screening in foods using a high data acquisition speed MS/MS

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Keywords:
LC/MS/MS, Pesticides Screening, Generic Peak Integration Parameters

Novel aspects:
Pesticides in food matrices were automatically identified by using fast 5 msec dwell time SRM and generic peak integration parameters

Abstract:
Effective management, use, and disposal of agrochemicals, particularly pesticides, is an increasing important health and environment issue in developing countries where economies may be heavily reliant on agriculture. The conventional approach is to develop highly optimized triple quad MRM methods to achieve the required levels of sensitivity and selectivity whilst still providing confidence in pesticide identification. The technology developed for fast scanning MRM analysis creates the advantage of developing multi-screening approaches for environmental target analysis and allows the possibility of a single generic 'universal' method. In this present study, high speed MRM analysis and a generic parameter set were used for screening 176 pesticides (352 SRM transitions) with 5 msec dwell and 1 msec pause times in different food matrices.

Compounds were analysed with a triple quadruple mass spectrometer (LCMS-8040, Shimadzu Corporation, Japan) . Separation was achieved with UHPLC (Nexera, Shimadzu Corporation, Japan) . 352 MRM transitions were monitored over the entire chromatographic run, 0 minutes to 20 minutes ; therefore retention time data was not required for each compound. The dwell and pause time for each SRM transition was 5 msec and 1 msec, respectively, including one polarity switch during the loop. The total loop time was 2.058 seconds which allowed approximately 10 data points to be collected for each peak, with a peak width for studied compounds of about 20 seconds.

Three different challenging matrices were analysed (green tea leaves, leeks and paprika) , with sample preparation carried out according to methods suggested by Japan's Ministry of Health, Labour and Welfare. 10 pesticides (Carbofuran, Chlorfluazuron, Fosthiazate, Hexathiazox, Indanofan, Lufenuron, Mevinphos, Propoxur, Pyrimidifen, and Tricyclazole) were spiked in green tea leaves, leeks and paprika extract solution, each at a 10 ppb concentration. A 100 ppb spiked sample (green tea leaves) was also prepared to develop the generic peak integration parameters used for compound identification. For each MRM mass chromatogram, the largest peak was automatically selected as the target compound to permit automatic identification of target analytes without retention time data.
The results showed that with regards to leek and paprika extract all 10 pesticides were automatically detected. In the case of a green tea leaf extract, all pesticides except Indanofan were correctly identified (Indanofan was not automatically identified as a larger peak was also present in the mass chromatogram ; a minor change to the method to create a retention window would resolve this issue) .
The screening of 176 pesticides resulted in eight false positives in leek extract, seven in paprika extract and ten in green tea leave extract.

PTh-176
13:30 – 14:40

Multi-class pesticide analysis in challenging vegetable matrices using fast 5 msec MRM with 15 msec polarity switching

Yuka Fujito[1], Yusuke Inohana[2], Kiyomi Arakawa[2], Hirano Ichiro[2]

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Keywords:
Pesticide, QuEChERS, LCMS-8040 triple quadrupole mass spectrometer, Ultra-fast MRM, Ultra-fast polarity switching

Novel aspects:
Over one hundred pesticides spiked into complex vegetable matrices at 5 ppb were successfully detected in a single analysis.

Abstract:
Many regulatory authorities have established multi-class residual pesticides methods for the analysis of vegetables, fruits and other food stuffs. However, there is no global agreement on the provision of a target list of pesticides and this presents a risk with products moving between different regulatory requirements.
In order to eliminate this risk, food safety laboratories need to ideally screen as many compounds as possible in a single run which may reach maximum residual limits (MRL) ; typically 10ppb in food matrices. In this study we report the application of ultra-fast 5 msec SRM with 15 msec polarity switching for the analysis of 138 pesticides, while still obtaining excellent LOQs for the majority of compound < 10 ppb.
Leek and Paprika (yellow and red) were purchased from a local Japanese grocery store, with the country of origin Japan (Leek) and New Zealand (Paprika) . Sample preparation was carried out by the use of a quick, easy, cheap, effective, rugged and safe (QuEChERs) method. LCMS-8040 triple quadruple mass spectrometer (Shimadzu, Japan) was used to monitor 276 MRM transitions (two MRMs per compound) . Each transition was monitored plus-minus 0.8 minutes of the target analytes retention time. The maximum loop time was 0.58 seconds and the maximum number of overlapped MRM transitions was 98 (Dwell time : 5 msec, Pause time : 1 msec) .
According to the method published by the European Union Reference Laboratory (EURL) entitled "Multiresidue Method using QuEChERS followed by GC-QqQ/MS/MS and LC-QqQ/MS/MS for Fruits and Vegetables ", 138 pesticides were selected as target analytes with sample pre-treatment by QuEChERs methods. Of these 138 pesticides, the EURL analysed and validated 66 pesticides by GC-QqQ and the remaining 72 pesticides by LC-QqQ. In contrast to the EURL method, in this study all 138 pesticides were analysed in a single analysis using MRM after spiking into vegetable matrices. This was achievable due to the fast dwell (5 msec) and pause times (1 msec) and rapid polarity switching (15 msec) of the triple quadruple mass spectrometer. Each MRM was optimised and calibration curves constructed in the range 1 to 1000 ppb. 21 pesticides measured by GC-QqQ in the EURL method did not reach the necessary LOQ (10 ppb) , but the remaining 45 pesticides achieved excellent LOQs in the range 0.08 to 10 ppb. 5 ppb and 50 ppb matrix matched standards were prepared for recovery studies. 90 % of the pesticides analysed presented excellent recoveries in the range 70 -120 % in all studied matrices.

PTh-177
11:10 – 12:20

High Throuput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS

Masayoshi Tamura[1], Keiko Matsumoto[2], Jun Watanabe[2], Junko Iida[2], Yasushi Nagatomi[1], Naoki Mochizuki[1]

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Keywords:
mycotoxins, carry over, high throughput, beer-based drinks

Novel aspects:
Development of a robust MS/MS method for the analysis of mycotoxins in complex commercial beverages with near zero carry over.

Abstract:
Mycotoxins often exist as contaminants in grains used in beer manufacturing. To maintain consumer needs for food safety, highly sensitive methods of detection are required by food and beverage manufactures.

UHPLC-MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. A high throughput method for the quantification of 14 mycotoxins in beer-based drinks was developed. While high sensitivity analysis was achieved, the nature of the analysis is characterized by a significant carry over issue as a result of the complex matrix. To enhance the robust detection of mycotoxins in beer-based drink analysis and to maintain high sensitivity, a novel autosampler technology was evaluated in this study.

MRM transitions were optimized for a range of mycotoxin standards (patulin, nivalenol, deoxynivalenol, aflatoxin B_1, B_2, G_1, G_2, T-2 toxin, HT-2 toxin, zearalenone, fumonisin B_1, B_2, B_3, and ochratoxin A) and analyzed using a Nexera UHPLC coupled to LCMS-8030 triple quadrupole mass spectrometer (Shimadzu Co.) . Mycotoxins were separated using ODS column maintained at 40 °C. Mobile phase : A - water + ammonium acetate, B - methanol + acetic acid. LC/MS was equipped with an electrospray ion source, and analyzed in positive / negative switching mode.

Nexera autosampler was configured to perform multiple rinse modes with two rinse solvents to eliminate sample carry over.

To optimize MS detection, the MRM method for the 14 mycotoxins took into account positive and negative ion detection ; Aflatoxin B_1, B_2, G_1, G_2, T-2 toxin, HT-2 toxin, fumonisin B_1, B_2, B_3, and ochratoxin were detected in positive mode while patulin, nivalenol, deoxynivalenol and zearalenone were detected in negative mode.

UHPLC separation optimization for fumonisin B_1, B_2, B_3 was enhanced with acetic acid as the buffer solution. Conversely, acetic acid solution in the mobile phase reduced the sensitivity for patulin, nivalenol and deoxynivalenol. By controlling the concentration of acetic acid and ammonium acetate with gradient elution program, the response and chromatographic behaviour for 14 mycotoxins was optimized using a cycle time of 11 minutes.

However, autosampler carryover was observed for fumonisin B_1, B_2, B_3 and zearalenone using a single rinse mode. To eliminate this problem completely a novel auto-sampler technology was considered. Using the multi-rinse function of SIL-30AC auto-sampler (Shimadzu Co.) , the interior and exterior surfaces of the autosampler needle were automatically washed with 2 different types of rinse solutions. In this auto-sampler mode, the dilution series of 14 compound standards resulted in linear calibration curves, excellent sensitivity near zero carry over. This method was then applied to the routine quantitative analysis of mycotoxins present in a variety of commercial beer-based drinks following extraction and purification by SPE.

PTh-178
13:30 – 14:40

Molecular Imprinted Polymer@Magnetic Nanoparticles Combined with Liquid Chromatography Mass Spectrometry for Determination of Glycoalkaloids in Potato

Cheng-Hsin Yeh, Maw-Rong Lee

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Keywords:
molecular-imprinted polymer, magnetic nanoparticles, liquid chromatography-mass spectrometry, food safety, toxic glycoalkaloids

Novel aspects:
MIP@MNPs coupled with LC-MS for determination of trace toxic glycoalkaloids in potato plants.

Abstract:
Some of the glycoalkaloids in food have toxicity to humans. Among the toxic glycoalkaloids； α-solanine and α-chaconine are produced in all port of the potato plants. In food safety issue, it is important to develop a sensitive and selective analytical method for quantification of trace toxic glycoalkaloids in food. In this research, a novel analytical method, molecular-imprinted polymer-modified magnetic nanoparticles (MIP@MNPs) as extraction adsorbents combined with liquid chromatography-mass spectrometry, was developed for determination of trace α-solanine and α-chaconine in potato plants and relative products. The experimental conditions including the synthesis of MIP@MNPs and extraction parameters were optimized in this study. The linearity, correlation coefficient, limits of detection (LODs) , and limits of quantitation (LOQs) were also evaluated. The analytical linear ranges for α-solanine, α-chaconine, and solanidine were obtained at 0.01-10 and 0.05-50 ng/g, respectively. The precision expressed by relative standard deviation (RSD) were below 8.2%. The LODs of proposed method ranged from 11 to 160 pg/g. The feasibility of applying the proposed method to determine the trace toxic glycoalkaloids in potato was also examined. The trace amount of α-solanine, α-chaconine, and solanidine in potato were detected by proposed method at 3.3, 6.0, and 4.2 ng/g, respectively. The results showed the developed method can be successfully utilized for analyzing the α-solanine, α-chaconine, and solanidine in potato.

Poster Session

Thursday, 20th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Food safety

PTh-179

11:10 – 12:20

Determination of cyromazine and melamine in chicken eggs by using QuEChERS coupled with liquid chromatography tandem mass spectrometry

Pei-Cheng Wang, Ren-Jye Lee, Chi-Chung Chou, Maw-Rong Lee

National Chung Hsing University, Taichung, Taiwan

Keywords:

Cyromazine, melamine, QuEChERS, LC-MS/MS

Novel aspects:

QuEChERS was developed to extract the trace cyromazine and melamine in chicken egg samples by using small volume of extraction solvent and variety of sorbent.

Abstract:

A rapid and sensitive method has been developed for the simultaneous detection of cyromazine and melamine in chicken eggs by using the quick, easy, cheap, effective, rugged and safe (QuEChERS) method coupled with a liquid chromatography tandem mass spectrometry (LC-MS/MS) system. The optimal sample preparation conditions for liquid-liquid extraction were 5 mL of acetonitrile with a 0.1 M hydrochloric acid aqueous solution (99.5 : 0.5, v/v) . The extract was cleaned with 0.5 g of anhydrous magnesium sulfate and 10 mg of graphitized carbon black. The analysis of cyromazine and melamine was accomplished by combining the use of an anion exchange LC column with tandem mass spectrometry in the positive electrospray ionization with selected reaction monitoring mode (SRM) . The detection limits were 1.6 ng g^{-1} for cyromazine and 7.5 ng g^{-1} for melamine, and the quantification limits were 5.5 ng g^{-1} for cyromazine and 25 ng g^{-1} for melamine. The recovery of cyromazine and melamine in spiked egg sampleswas 83.2 % and 104.6 % , respectively, with an RSD of less than 18.1 % . Intra-day and inter-day precision, represented as an RSD ranged from 1.5% to 8.8% and 6.8% to 14.3%, respectively. The proposed method was tested by analyzing chicken eggs from the market and the veterinary medicine laboratory. The results of this study reveal that the proposed method can be used to adequately analyze cyromazine and melamine in chicken eggs. The concentrations of cyromazine and melamine detected in these samples were in range of 20 to 94 ng g^{-1}.

PTh-180
13:30 – 14:40

DIFFERENTIATION OF FARMED FISH AND WILD SEAFISH USING ISOTOPIC SIGNATURES

Lian Jie Bay[1], Jaime Wong[1], Daniel Fliegel[2], Sheot Harn Joanne Chan[3], Thomas R Walczyk[1]

[1]National University of Singapore, Singapore, [2]National Institute of Nutrition and Seafood Research, Bergen, Norway, [3]Health Sciences Authority, Singapore

Keywords:
Strontium Isotopic Ratios, Light Isotopic Ratios, Farmed VS Wild Marine Fish, Atlantic Cod, Food Fraud

Novel aspects:
First report on the use of isotopic tecnniques to differentiate between wild and farmed atlantic cod, particularly the use of strontium isotopic ratios which has never been attempted.

Abstract:
Worldwide consumption of fish has risen dramatically over the decades. Overfishing, a consequence of this demand, has led to decline of fish stocks and depletion of marinefood webs in many parts of the world. To meet the increasing demand for seafood, aquaculture has developed as it offers a cost effective supply of seafood. However, aquaculture employs the use of antibiotics, dyes, growth hormones, "sustainable fish feeds " where marine proteins are being substituted with plant based proteins as well as fish-feed produced from genetically modified raw materials. This may result in increased levels of contaminants in aquacultured fish but also may change their nutritional value. Wild fish being perceived as the superior product can therefore command a higher price on retail markets. This opens avenues for food-fraud by mislabeling. It is estimated that the origin of 25 to 70% of seafood is fraudulently declared. This project aims to develop an ultrasensitive method to distinguish wild and farmed cod (*Gadus morhua*) by employing strontium ($^{87}Sr/^{86}Sr$) , carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios in fish meat as a dietary marker. Techniques involving Thermal Ionisation Mass Spectrometry (TIMS) and Isotope Ratio Mass Spectrometry (IRMS) for measuring carbon, nitrogen and strontium isotope variations were developed.

Differences in natural strontium isotopic ratios arise in nature due to the decay of ^{87}Rb to ^{87}Sr. This radiogenic decay has produced distinctly different Sr isotope abundances in different parts of the Earth crust over its history. This isotopic signature is incorporated into plants and will be passed onto fish through feed prepared from the plant material. In contrast, due to the long residence time of Sr in seawater (millions of years) , compared to the turnover time of the oceans (millennia) , $^{87}Sr/^{86}Sr$ is homogeneous throughout the world's oceans at any given time. Wild marine fish should display the unique strontium isotopic ratio of seawater as a result of feeding solely on marine organisms, while farmed fish should exhibit an isotopic ratio more terrestrial in nature, as fish feed in aquaculture utilizes terrestrial plant based materials. Differences in carbon and nitrogen isotopic values arise for the same reason, i.e. wild and farmed cod should differ essentially in the isotope signature taken up from feed.

Methods for the analysis of strontium isotopic ratios in cod involved microwave digestion of tissue, separation of the strontium ions from interfering elements using ion exchange chromatography and finally analysis of the purified fraction using TIMS. Achievable precisions for $^{87}Sr/^{86}Sr$ are of the order of parts per million (0.0017% RSD ; n= 6) . Analysis of both wild and farmed cod from Norwegian fish farms showed similar strontium isotopic ratios despite differences in feed. This contradicts the initial hypothesis that both groups of fish should exhibit different strontium isotopic ratios and that cod acquires strontium primarily from water and not from feed. Measurement precision was on the order of 0.1‰ for nitrogen and 0.06‰ for carbon. By combining both signatures, wild and farmed cod could be distinctly separated from one another which may turn the developed techniques into an important tool for differentiating farmed from wild cod.

PTh-181
11:10 – 12:20

Evaluation of a new ion source to improve the limit of quantitation for chloramphenicol on a UHR-TOF

Karin Wendt, Thomas Zey, Amalia Apalategui, Carsten Baessmann, Peter Brechlin

Bruker Daltonik GmbH, Bremen, Germany

Keywords:
Heated electrospray source, 15 to 15 fold improvement in sensitivity, quantification of chloramphenicol in different matrices

Novel aspects:
Improved sensitivity for chloramphenicol in different matrices on a UHR-TOF with a newly developed heated electrospray source

Abstract:

Introduction

Chloramphenicol (CAP) is used as a broad-spectrum antibioticum in veterinary medicine. Due to the serious side effects for humans it has been banned for all food producing animals in various countries. Current law in the European Union (EU) requires a minimum required performance limit (MRPL) of 0.3 ug/kg or 0.3 ppb. We present here a work using an accurate mass target screening approach to quantify CAP in different typical matrices, such as meat, urine or honey. Heated electrospray sources are known to be show sensitivity boosts for certain compound classes. A newly developed heated electrospray source will be presented that is capable of high liquid flows and should help to improve the sensitivity of these samples from complex matrices.

Methods

Chloramphenicol was spiked into homogenized beef-muscle at different concentrations from 0.25 ng/kg to 10 ug/kg. For sample preparation ethylacetate was added. The sample tubes were vortexed, shaken overhead, centrifuged and the supernatant collected. The combined ethylacetat-fraction was applied to equilibrated NH 2 SPE (Supelco supelclean NH 2 500 mg, 3 mL) and the eluate directly collected. The samples were separated with a short 4.5 min gradient on a UHPLC system. Data acquisition was performed in negative mode using a Ultrahigh Resolution (UHR) -TOF equipped with a new heated electrospray ionization MS source. For comparison the samples were analyzed with a standard ESI source and the heated ESI source.

Preliminary Data

During the characterization of the source it was identified that important parameters for the heated electrospray are sheath gas and the vaporizer temperature. For chloramphenicol the optimum conditions were 4 l/min N 2 sheath gas and 320 degree C vaporizer temperature. With the new heated electrospray a 10 to 15 fold improvement in sensitivity was observed for chloramphenicol in the different matrices, compared to a standard electrospray source. In meat the limit of detection was below 0.001 ug/kg and the limit of quantitation below 0.0025 ug/kg. At 0.0025 ug/kg the average S/N was 15.4 and the RSD 9 % for 6 technical replicates. We found a good linear dynamic range from 0.001 to 10 ug/kg. The limit of quantitation for this zero-tolerance substance in the different matrices was well below the required MRPL of 0.3 ug/kg. The complete characterization of the source for the quantification of chloramphenicol in different matrices will be presented.

PTh-182
13:30 – 14:40

MASS SPECTROMETRY IDENTIFICATION AND CHARACTERIZATION OF GIBBERELLINES IN FRUITS

ENCARNACION MOYANO, GABINO BOLIVAR-SUBIRATS, HECTOR GALLART-AYALA, MARIA TERESA GALCERAN

Barcelona University, Barcelona, Spain

Keywords:
Gibberellins, UHPLC, Mass Spectrometry, Electrospray

Novel aspects:
A common fragmentation pathway for GAs is established. A strategy for non-target GAs identification using mass spectrometry data and LC retention windows predicted from estimated log Poct/wat is proposed

Abstract:
Gibberellins (GAs) are an important class of plant hormones that regulate growth and influence some developmental processes. GAs are used in agriculture and horticulture to break dormancy, to speed up flowering and fruiting and to stimulate the production of seedless fruits in the absence of pollination, probably by stimulation of production of endogenous GAs that enhance their activity. All known GAs are tetracyclic diterpenoid acids derived via the ent-gibberellane skeleton,but synthesised via ent-kaurene. Actually, more than 130 GAs have been identified from plants, fungi and bacteria and they are classified in two classes based on the presence of either 19 or 20 carbons. The 19-carbon gibberellins, such as gibberellic acid (GA 3) , possess a five-member lactone bridge that links carbons 4 and 10. The 19-carbon forms of GAs are the biologically active. These compounds differ in the number and site of hydroxyl groups. In general, the most biologically active compounds are dihydroxylated gibberellins, which possess hydroxyl groups on both carbon 3 and carbon 13 (e.g. GA 3) . The low concentration of some of these compounds and the matrix complexity of vegetable samples make indispensable the use of mass spectrometry in combination with liquid chromatography (LC/MS) for their identification, confirmation and characterization. It must be taken into account that the few commercial standards available make difficult the development of analytical methods. Additionally, tandem mass spectrometry information about GAs is limited.
The aim of this work was to develop a sensitive and selective method for the identification and characterization of GAs. For this goal, mass spectrometry tools and chromatographic strategies have been combined to propose a method for the analysis of the whole family of GAs. Ultrahigh performance liquid chromatography on a sub-2 μm C18 reversed phase column (MeOH : 0.1 % formic acid and gradient elution) provided the separation of available standards (GA1, GA3, GA 4 and GA 7) in less than 5 min with high efficient peaks. Octanol-water constants (log $P_{oct/wat}$) were estimated (software : VCCLABS) for all GAs and their interpolation in the experimental retention data allowed us to predict retention windows for the searching of non-target GAs. Electrospray was used as ionization source in negative mode being $[M\text{-}H]^-$ the base peak. A fragmentation pathway was proposed for GAs based on the tandem mass spectrometry data obtained in a triple quadrupole mass analyzer and this fragmentation pathway was used to predict the potential main product ions of non-target GAs. The diagnostic ions were used for the tentative identification of non-target GAs in fruit samples (apple, pear and peach) collected thorough the whole growth period. The presence of these GAs was confirmed by using accurate mass measurements on an Orbitrap mass analyzer and product ion scan with reversed energy ramp (RER) on a triple quadrupole instrument was used to monitor product ions of both low and high molecular weight. Target GAs (GA1, GA3, GA 4 and GA 7) and some non-target GAs (e.g. GA9, GA10, GA21, GA82) were detected and confirmed in these samples.

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- SofwareVCCLABS for log $P_{oct/wat}$estimation : www.vcclab.org/lab/alogps

PTh-183
11:10 – 12:20

EVALUATION OF ATMOSPHERIC PRESSURE IONIZATION SOURCES FOR MASS SPECTROMETRIC ANALYSIS OF PHENICOL DRUGS

ELIDA ALECHAGA, ENCARNACION MOYANO, MARIA TERESA GALCERAN

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Keywords:
Phenicols, ESI, APCI, APPI, tandem mass spectrometry

Novel aspects:
Comparison of the behaviour of phenicols in API sources (ESI, APCI, APPI) is performed. Mass spectrometry fragmentation pathway has been established and some product ions has been corretly assignmed

Abstract:
Nowadays, LC-MS has become a robust and well-established analytical technique for the analysis of small molecules in food, biological and environmental matrices. Until recently, the most commonly used atmospheric pressure ionisation (API) sources for coupling liquid chromatography to mass spectrometry have been electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) [1, 2], but atmospheric pressure photoionisation (APPI) has gained popularity in the last few years expanding the application field of LC/MS [3]. However, there are only a few studies in which the suitability of the ESI, APCI and APPI sources for the analysis of veterinary drugs in food matrices has been compared [4-6].
In this work, the veterinary drug family of phenicols, including chloramphenicol (CAP), a wide-spectrum antibiotic, and its related compounds thiamphenicol (TAP), florfenicol (FF) and florfenicol-amine (FFA), was studied by LC-MS/MS. Their behaviour in ESI, APCI and APPI under fast liquid chromatographic separation conditions (Fused Core Phenyl-Hexyl column, methanol : acetic acidammonium acetate buffer, 5 mM and pH 5) was compared taking into account both positive and negative ionization modes. For APPI different dopants (toluene, anisole, tetrahydrofuran, chlorobenzene and acetone) were tested evaluating their performance in terms of ionization efficiency and mass spectral data. In all API sources CAP, TAP and FF ionized in both positive and negative modes, while for the metabolite FFA only positive ionization was possible. In general, in positive mode $[M+H]^+$ dominated the mass spectrum for FFA, while the other compounds, CAP, TAP and FF, with lower proton affinity showed an additional intense ion $[M+NH_4]^+$. Moreover, in-source fragmentation was also observed (loss of H_2O) for FFA, FF and CAP at high source temperature such as in H-ESI and APCI. This water loss was enhanced in APPI and the relative abundance of this fragment ion depended on the dopant nature. In some cases, it become the base peak of the mass spectrum. In negative mode, ESI and APPI showed the deprotonated molecule $[M-H]^-$, while APCI provided the radical molecular ion by electron capture. Additionally, chloride adduct formation $[M+Cl]^-$ was observed in ESI and APPI (depending on the dopant) and the loss of HF occurred. All these ions were characterized by tandem mass spectrometry a common fragmentation pathway was established for this family of compounds. This mass spectrometry study allowed us to clarify some discrepancies observed in the literature in relation to the assignment of some product ions commonly used for quantitative and confirmative purposes.

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PTh-184

13:30 – 14:40

High-throughput simultaneous analysis of pesticide residues in food by supercritical fluid chromatography/tandem mass spectrometry

Megumi Ishibashi[1], Ando Takashi[2], Sakai Miho[2], Matsubara Atsuki[1], Uchikata Takato[1], Fukusaki Eiichiro[1], Bamba Takeshi[1]

[1]Osaka University, Osaka, Japan, [2]Miyazaki Agricultural Research Institute, Miyazaki, Japan

Keywords:
pesticide residues analysis, supercritical fluid chromatography, mass spectrometry

Novel aspects:
Supercritical fluid coupled with tandem mass spectrometry allowed us to analyze pesticides with a wide range of polarities and molecular weights simultaneously.

Abstract:
Application of pesticides to agricultural products is an efficient means of mitigating unacceptable loss of agricultural production. However, pesticide residues in food products may cause health problems ranging from simple skin irritation and nausea to more life-threatening ones such as acute poisoning and breathing problems. Therefore, the maximum residue limits (MRLs) for pesticides, veterinary medicines, and food additives have been established in various countries. Hence, many researchers have focused on the development of a system for the simultaneous and rapid analysis of multicomponent pesticides. Combination techniques such as gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) are commonly used for pesticide residue analysis, but there is no reported method for the simultaneous analysis of multiple pesticides in a sample using a single instrument. Supercritical fluid chromatography (SFC) offers high resolution at high flow rates and various separation modes and hence may aid the rapid simultaneous analysis of pesticide residues. We developed an SFC/MS/MS method and analyzed 17 pesticides with a wide range of polarities (log*P*ow = 4.6 to 7.05) and molecular weights (112.1 to 888.6) within 11 min using a polar-embedded reverse-phase column. This SFC-based system can analyze various pesticides that are traditionally analyzed by separate processes such as GC/MS and LC/MS. Since the elution power of SFC can be changed by the addition of a polar solvent, diquat dibromide together with cypermethrin and tralomethrin could be detected in the presence of various other pesticides using a single mobile phase. Usually, diquat dibromide requires a special analysis method such as ion chromatography and ion-pair LC, while cypermethrin and tralomethrin are analyzed by GC. This result showed that SFC is also applicable to the analysis of many more pesticides with log*P*ow values ranging from 4.6 to 7.05. Most of the compounds were detected even at ng/L levels, while diquat dibromide, fosetyl, maleic hydrazide, and cypermethrin were detected at µg/L levels. The limit of detection (LOD) and limit of quantification (LOQ) of most compounds were higher in agricultural extracts (rice, onion, and spinach) than in standard mixtures. However, the LODs of most compounds except for highly hydrophobic ones such as tralomethrin, cypermethrin, and etofenprox in onion were much lower than the corresponding Japanese maximum residue limits. The proposed method can also be used to detect pesticide residues in agricultural product extracts obtained by a suitable extraction method ; the sample purification procedure can be simplified by adopting the standard addition method, and the analysis does not involve any complex pretreatment process such as derivatization. Therefore, we conclude that SFC/MS is a high-throughput, robust, and highly reproducible technique for rapid and simultaneous pesticide residue analysis. A more practical screening system based on SFC/MS for pesticide residues will be constructed in near future.

PTh-185 Chromatographic Separation of Chrysene and Triphenylene in Smoked Fish

11:10 – 12:20

Mirja L Hokkanen, Marika N Jestoi, Kimmo E Peltonen

Finnish Food Safety Authority Evira, Helsinki, Finland

Keywords:
PAH, Separation, Chrysene, Triphenylene, GC-MS/MS

Novel aspects:
Recently GC columns that allow separation of chrysene and triphenylene were commercialised. A lack of chromatographic resolution might lead to overestimation of chrysene and to false conclusions of the samples.

Abstract:
Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants formed primarily by incomplete combustion during food processes, such as drying, grilling, smoking and roasting. The European Union (EU) has published a recommendation for the monitoring of 15+1 EU priority PAH compounds typically abundant in different kinds of foods. According to the scientific opinion by the European Food Safety Authority (EFSA, 2008) , benzo [a] pyrene alone is not a suitable indicator for the occurrence and toxicity of PAHs in food. Based on this fact as of September 2012, in addition to benzo [a] pyrene, the maximum limits will be determined for the sum of four PAHs (benzo [a] pyrene, benz [a] anthracene, chrysene and benzo [b] fluoranthene) .Typically the GC columns used for the separation of PAH compounds include non-polar stationary phases, developed for the determination of environmentally important PAHs, as reported in the US EPA priority list. However, these phases do not usually provide adequate separation for certain critical pairs of PAH's that are important for food contamination. An example of such critical pairs is chrysene (Chr) and triphenylene (Trp) . Chr and Trp are isomeric compounds having exactly the same molecular weight and fragmentation patterns, making their identification a challenging task even when using tandem mass spectrometry (MS/MS) . Their resulting co-elution, together with the fact that they are undistinguishable in the detector, may cause analytical problems such as overestimation of contamination levels for Chr, as well as for the PAH 4 compounds. Due to analytical problems for the determination of the critical pair of Chr and Trp by using traditional non-polar GC columns, other approaches must be applied to ensure consumer safety. Not until recently, did columns that allow separation of Chr and Trp become commercially available. In this study, GC columns with different chemistries were tested to solve the problem with the co-elution of Chr and Trp. The analytical columns tested were a Phenomenex Zebron ZB-50 and an Agilent Select PAH. The Zebron ZB-50 column has high polar phenyl siloxane phase which has higher temperature limits than other widely used stationary phase chemistries. The Select PAH capillary column offers enhanced selectivity towards PAHs, separating the isomers and overcoming the limitations of other GC columns. Smoked fish samples were analysed by using both columns. In addition, a sample with known amount of Chr obtained from an inter-comparison study was analysed. The determination of PAHs was conducted by using an ISO 17025 accredited GC-MS/MS method. In the poster presentation the effects of using those two GC column chemistries on the analytical results of Chr in fish samples will be presented. Preliminary findings from this study show that successful separation of Chr and Trp was achieved only with the Agilent Select PAH column.

PTh-186
13:30 – 14:40

Assessing the impact of a novel ion source design in food safety applications using MS/MS detection

Neil J Loftus[1], Simon Hird[2], David Baker[1], Alan Barnes[1]

[1]Shimadzu, Manchester, UK, [2]FERA, York, UK

Keywords:
Food safety, LC/MS/MS, data quality

Novel aspects:
Applying a novel ion source design with turbulent and laminar flow regions to food safety analysis using MS/MS detection

Abstract:
In today's global marketplace, as foods are produced and distributed throughout the world, food quality and food safety become increasing concerns for consumers, governments and producers. There is a need to develop residue methods with fast turn-around times and preferably with only minimal sample preparation and clean up. LC-MS/MS is the ideal analytical tool to fulfil the requirements for speed, sensitivity, selectivity and robustness. Recent developments in the design of mass spectrometer and LC column packing materials allow more components to be analyzed in the same injection within a reasonable short time and thus make high throughput multi-compound analyses become a reality. Reports of the LC-MS/MS determination of hundreds of pesticides in various fresh produce and other agricultural commodities and the determination of veterinary drug residues in animal tissues are now common place [[1]] .

Difficulties remain when trying to discriminate analytes from co-extracted matrix components that exhibit similar physiochemical properties. If not addressed this can lead to a reduction in quality of data and can impact a laboratory's performance and reputation. Matrix components can cause increased chemical noise and ion suppression. When experienced in combination this makes determinations of residues at low concentrations very difficult. In addition to problems caused by the sample matrix, some analytes exhibit poor response in electrospray ionisation. Successful analysis of these compounds to the regulatory concentration limits is difficult. Increasing the amount of sample introduced is impractical as this approach can lead to an increase in ion suppression. A much more practical solution is to use increased instrument sensitivity to maximise performance at these required concentrations.

This paper describes the application of a novel ion source design which increases electrospray sampling efficiency using turbulent and laminar flow gas flows generating ions with well-defined ion energy. Described here are some initial data illustrating the application of a novel source design interfaced with a triple quadrupole mass spectrometer to provide suitable sensitivity and robustness for the determination of pesticides and veterinary drug residues in a range of typical foodstuffs and animal tissues, respectively, for official control purposes.

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PTh-187
11:10 – 12:20

Development and applications of a pesticide multiresidue analysis turn-key system utilizing UHPLC-Orbitrap MS and post data processing

James Chang[2], Jon Wong[3], Kai Zhang[3], Serei Thach[4], Paul Yang[4], Charles Yang[2], Kristi Akervik[2], Maciej Bromirski[2], Yoko Yamagishi[1], Dipankar Ghosh[2]

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Keywords:
food, pesticide, contaminates

Novel aspects:
UHPLC-Orbitrap based multiresidue method has been shown to have equivalent performance in quantitative and superior ability in target and unknown identification.

Abstract:
Introduction

Multresidue methods are employed to monitor chemical contaminants in food and environmental samples and quantitate their concentrations to ensure that concentrations present do not exceed the tolerance levels established by government regulations. Many of these multiresidue methods have been developed for pesticides, mycotoxins, veterinary drugs, and polar organic pollutants employing UHPLC-MS/MS in targeted analysis. These methods are selective, sensitive, cost-effective and can be optimized to analyze as many target analytes in a single injection. However, optimization of MS/MS parameters is a very time consuming process ; and requires re-optimize the duty cycle, scanning efficiency and MRM transitions whenever new pollutants are added. Difficulties associated with maintaining MS/MS based method make UHPLC-Orbitrap and cheminformatic based multiresidue methods an attractive alternative. (119 words in total)

Methods

Samples including fresh produce and other food products such as wheat flour and raisin were purchased locally as organic or conventional products. Incurred samples were obtained from the FDA field laboratories and the EPA Analytical Chemistry Laboratory, Ft. Meade, MD. QuEChERS procedure was used in the preparation of method validation and incurred samples. Reversed phase UHPLC separation was achieved using 2.1x100 mm column at various flowrates to characterize and optimize Orbitrap operation parameters. All data were acquired in positive ionization mode. Full scan, high resolution MS data using a second generation Exactive were acquired at four different MS resolutions and various concentrations to investigate the selectivity and sensitivity. Acquired data were transferred and processed off-line using Exact Finder to data mining software to maximize operation efficiency. (119 words in total)

Preliminary data

Analytical data were obtained from a mixture of > 200 target pesticides prepared in solvent and produce sample matrices. These data are used for the determination of instrument detection limit (IDL) and matrix specific method detection limit (MDL) . Analyzed and processed at 5 - 7 levels of concentrations in 5 - 8 replicates analyses, the data shows that optimized Orbitrap extracted ion chromatogram (XIC) data can be collected at an resolution of 70,000 (FWHM at m/z 200) or higher as long as there is a minimal of 5 - 7 data points to define a specific chromatographic peak. IDLs were determined from solvent analytical standards and were in the high part-per-trillion to low part-per-billion range with > 90% of pesticides evaluated showed a MDL of 5 part-per-billion or less. Incurred samples analyzed such as orange, Kale and cabbage showed the presence of various pesticides that matched patterns of field application and usage very well. Quantitative data obtained from UHPLC-Orbitrap analysis showed an excellent equivalency compared to those obtained from MS/MS analysis using two MRM transitions. Orbitrap analysis did have an edge over the two MRM based MS/MS analysis in avoiding false-positive identification, especially chemical analogs like spinosyn A, C and D. Post data processing software provided for the identification of targeted unknowns using chromatography retention time, accurate mass and isotopic pattern ; achieving the identification and confirmation requirements in analytical chemistry. Additional cheminformatic software allowed for the use of ChemSpider of Royal Chemistry Society, greatly increase the confidence in the identification of unknown.

PTh-188

13:30 – 14:40

Multi-residue method for the confirmation of six avermectin residues in food products of animal origin by liquid-chromatogrphy tandem mass spectrometry

Myeong-Ae Kim, Jeong Woo Kang, Myung-Sin Lim, Young Hoon Bong, Chae-Mi Lim, Seoung-Wan Son

Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea

Keywords:

chromatography, LC/MS ; drug residues, ivermectin ; animal products, meat ; milk

Novel aspects:

The determination and develpement of multi-residue analysis method for the confirmation of six avermectin residues in food products of animal origin using QuEChERS sample preparation and liquid-chromatography tandem mass spectrometry

Abstract:

A confirmation method was developed for the rapid determination abamectin, ivermectin, doramectin, moxidectin, emamectin and eprinomectin residues in various food products of animal origin, such as pork muscle, pork liver, pork kidney, milk and egg. Samples were homogenized, extracted by acetonitrile, cleaned via dispersive-clean up procedure using magnesium sulfate and primary secondary amine (PSA) on based QuEChERS method (quick, easy, cheap, effective, rugged and safe) . All the six avermectin residues in different animal-food products were simultaneously separated and determined by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) within 10 min. Data acquisition under positive ESI-MS/MS was performed by applying multiple reaction monitoring (MRM) for both identification and quantification, and mass spectrometric conditions were optimized to increase selectively and sensitivity. The matrix-matched calibration curves for different matrices, such as pork muscle, pork liver, pork kidney, milk and egg, were constructed and the interference effect of different sample matrices on the ionization was effectively eliminated. The LC-MS/MS method was validated with satisfactory linearity, recovery, precision and stability. Matrix-matched calibration curves of abamectin, ivermectin, doramectin, moxidectin, emamectin and eprinomectin in five different matrices were linear (r20.990) in the range 2.52000 ug/kg. The limit of detection and quantification for the six avermectins were in the range 0.1-12.2 and 0.2-40.6 ug/kg, respectively. Recoveries were 62.9-120.9 % with good intra- and inter-day precision of 7.0-14.4 and 8.6-15.0%, respectively. The method was rapid, sensitive and reliable, and can be applied to the quantitative analysis of avermectin residues in different animal food products.

PTh-189
11:10 – 12:20

Multi-residue analysis of 59 pesticides in raw bovine milk using GC-MS/MS and UPLC-MS/MS

JinYoung Shin, Dong-Gyu Kim, Kyung Yuk Ko, MeeKyung Kim, Chae-Mi Lim, Seong-Wan Son

Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea

Keywords:
Pesticides ; Multi-residue analysis ; raw bovine milk ; GC-MS/MS ; LC-MS/MS

Novel aspects:
Multi-residue analysis of pesticides in raw bovine milk using QuEChERS method with GC-MS/MS and LC-MS/MS

Abstract:
A fast multi-residue method was developed for the screening, quantification and confirmation of 59 pesticides, belonging to different chemical families of insecticides, acaricides, fungicides, herbicides and insect growth regulators in raw bovine milk using QuEChERS method with GC-MS/MS and UPLC-MS/MS. The Optimization of different parameters such as extraction solvents (acetonitrile, acetone, or ethyl acetate) , type of clean-up sorbents (PSA, C18, or GCB) , GC and UPLC separation and MS/MS parameters were carried out. The developed method which used acetonitrile/ 1 % acetic acid as a extraction solvent and used PSA/C18 sorbents as a cleanup step was applied. Under the optimized conditions, GC and UPLC-MS/MS gave linear calibrations over the concentration range from 1 to 100 ng/mL ($r > 0.990$) . The recoveries were, with a few exceptions, in the range of 61 - 124 % at the different spiked levels with satisfactory precision ($CV < 16$ %) . The quantification of analytes was carried out using the most sensitive transition for every compound and by matrix-matched standards calibration. Limits of detection (LOD) and quantification (LOQ) varied from 0.1-9.2 ng/mL and 0.2-19.5 ng/mL, respectively. The results indicated that the proposed method can be applied to the determination of 59 pesticides which have been established MRLs (maximum residue levels) in raw bovine milk.

PTh-190

13:30 – 14:40

Untargeted Plasma Metabolomics using 13C-Glucose: Glycated-Metabolites as Novel Biomarkers for Aging and Diabetes

Daiki Setoyama, Yoshinori Fujimura, Hiroyuki Wariishi, Daisuke Miura

Kyushu University, Fukuoka, Japan

Keywords:

Glycation, Metabolite, Metabolomics, Plasma, 13C-Glucose

Novel aspects:

With our strategy, some of the glycated metabolites can be identified to be accumulated in aged and diabetic plasma, implying the potential relationship between the glycation of metabolites and diseases.

Abstract:

Highlights

Protein-glycation has been implicated in the pathogenesis of aging, diabetes, and other angiopathy-related diseases because it potentially produces advanced glycation end-products (AGEs) to cause intracellular oxidative stress and alterations in protein functions. However, little has been verified for glycation of metabolites in cells and their involvement in cellular dysfunctions. In this study, we performed LC-MS-based untargeted metabolomics for mouse plasma with an attempt to obtain a library of glycation-vulnerable metabolites that could react with ^{13}C-glucose *in vitro*. With the library, it is evaluated whether the glycated metabolites would be indeed found (or accumulated) in plasma derived from naturally-aged mice and disease-related mice, such as senescence accelerated-prone (SAMP 8) and diabetic db/db lineages, respectively. Interestingly, we observed that some of the glycated metabolites, including amino acids and peptides; lysine, glutamine, taurine, gamma-glutamyl-lysine and glutathione, were identified to be accumulated in db/db plasma as compared with control plasma. We could also detect two kinds of AGEs, carboxymethyl lysine (CML) and 3-deoxyglucosone (3DG) in diabetic plasma. Although the causal relationship between the glycated metabolites identified and their productivity of AGEs awaits further investigation, this study will provide some potential biomarkers for early diagnosis of aging and diabetes and also offer a useful annotating strategy for previously unregistered (in database) metabolites having specific chemical modification, like glycation.

Rationale

Glycation, a chemical reaction in which a reducing sugar is added to an amino group of proteins, occurs non-enzymatically in high glucose conditions or hyperglycemia. It results in the accumulation of deleterious advanced glycation endproducts (AGEs), some of which accelerate cross-linking reaction between proteins, thereby causing their aggregation, degeneration, and dysfunction. Since accumulation of AGEs is suggested to be involved in aging and deterioration of diabetes-related complications, it is critical to regulate generation of AGEs for maintaining intracellular homeostasis. Previous studies, however, have mainly focused on protein-glycation as an AGEs-generator, underestimating the significance of the glycation of small-weight molecules, metabolites, in cells. Given that some of the metabolites have amino group in their side chain and high reactivity with many bio-macromolecules such as proteins, nucleic acids, and lipids, it would be reasonable to say that metabolites can be glycated as well to serve as a source generating harmful AGEs or *per se* as a noise or trigger for intracellular signal transductions. Here, we perform a mass spectrometry-based metabolomics in combination with ^{13}C isotope labeling strategy to explore the glycated metabolites in mouse plasma. To our knowledge, this kind of comprehensive study would be unprecedented and we therefore believe that the knowledge obtained in this study would not only be beneficial for a better understanding of diseases but also lead to uncover novel aspects of molecular mechanism underlying intracellular homeostasis.

PTh-191
11:10 – 12:20

The examination of high-throughput, high-sensitive and more-ecological measurement by micro flow UHPLC/MS/MS system

Seiji Horie[1], Kazuhide Inoue[1], Masazumi Yasumoto[1], Kayoko Hagiwara[2], Shigeru Yamada[2], Yasutoshi Kawase[1]

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Keywords:
Micro-flow-UHPLC, LC/MS/MS, High-throughput, Needle-spray-voltage

Novel aspects:
High-throughput and high-sensitive LC/MS/MS analysis by using micro flow UHPLC

Abstract:
The measurement by LC/MS/MS is used at various fields, e.g., development of drugs, examination of food safety and so on. The sensitivity of the MS has been improving according to demand of the user year by year. However, further high sensitive device is required, and besides, high-throughput method is regarded as important. Recently high flow rate HPLC, UHPLC, is used very widely for LC/MS/MS analysis. But there is a demerit from viewpoint of disposal and environment load because of using a lot of organic solvent as mobile phase.
On the other hand, Nano LC is used in high-sensitive detection of small amount of biological substances. But operation of Nano LC is complicated and it is difficult to obtain stable results. So, it is not spread widely in the field using traditional HPLC methods.
In this report, we present the examples of high-throughput and more-ecological analysis by using "Micro flow UHPLC " specialized in the flow rate between Nano LC and traditional HPLC.
As Micro flow UHPLC, Eksigent ekspertTM microLC 200 was used. This system can analyze by flow rate range 5 to 50ul/min or 20 to 200ul/min respectively by changing the flow modules. Microfluidic Pump Control (MFC) technology in this system allows for rapid gradients to be run with excellent accuracy and reproducibility.
All experiments were run on an AB SCIEX QTRAP (R) 5500 LC/MS/MS system. To reduce band broadening at low flow rate, Hybrid electrode which has small diameter more than normal electrode was set to AB SCIEX QTRAP (R) 5500 LC/MS/MS system.
We also report the examination of the suitable voltage applied to the spray needle of ESI for various flow rate.

PTh-192

13:30 – 14:40

Screening analysis for drugs of abuse by LC-MS/MS enables fast polarity switching MRM triggered product ion scanning on the fly

Toshikazu Minohata[1], Ichiro Hirano[1], Junko Iida[1], Keiko Kudo[2], Noriaki Ikeda[2], Kei Zaitsu[3], Noriaki Shima[3], Munehiro Katagi[3], Hitoshi Tsuchihashi[4], Koichi Suzuki[4]

[1]Shimadzu Co., Kyoto, Japan, [2]Kyushu University, Fukuoka, Japan, [3]Osaka Prefectural Police, Osaka, Japan, [4]Osaka Medical Collage, Takatsuki, Japan

Keywords:
LC-MS/MS, forensic, drug screening

Novel aspects:
A new approach of drugs of abuse screening using simultaneous analysis method by LC-MS/MS

Abstract:
Introduction. In recent years, the needs of forensic, toxicological and clinical analysis are becoming increasingly challenging as a consequence of a growing trend in the use of illicit drugs and the non-medical use of prescription drugs. Screening applications require rapid and unambiguous results that can be achieved using a generic analysis method designed for a large number of target compounds. To meet this need a universal high speed MRM triggered product ion scanning method with fast polarity switching was applied to simultaneously screen, quantitate and confirm (by reference to a MS/MS data base) the majority of drugs of abuse available in Japan. **Methods.** Samples were measured using a Nexera UHPLC system and LCMS-8030 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) with MRM triggered automatic MS/MS data acquisition. Samples were separated using a standard clinical protocol on a Shim-pack FC-ODS column (150 x 2 mm, 3 um) . A flow rate of 0.3 mL/min was used together with a gradient elution [A ; 10mM ammonium formate in water, and B ; methanol] . **Results.** A high speed LC/MS/MS data acquisition system was applied to drug screening in forensic, toxicological and clinical analysis. To achieve a highly specific and sensitive detection in screening and quantitation a MRM triggered product ion scanning method using a polarity switching speed of 15msec and a scan speed of 15,000u/sec was applied to 111 components including illicit drugs, psychotropics, hypnotics, pesticides and other substances. As the MRM acquisition time was very fast, this enabled product ion spectra to be generated in both positive and negative ionization mode which could be matched against a user library of compounds as an automated aid to screening and compound identification. **Conclusions.** By bringing together optimized detection Synchronized Survey Scan™ parameters (product ion spectral data acquisition parameters based on the MRM intensity as threshold) and library matching into a single integrated method package helps to generate high quality data without the need for a complex start-up.

PTh-193
11:10 – 12:20

HPLC method scouting system using ultra high performance liquid chromatography coupled to single quadrupole mass spectrometer

Yusuke Inohana, Taku Tsukamoto, Hidetoshi Terada, Kiyomi Arakawa, Ichiro Hirano

Shimadzu Co., Kyoto, Japan

Keywords:

Method scouting, UHPLC, sensitivity (LC/MS), chromatographic separation

Novel aspects:

Within a day, high sensitive LCMS methods were developed for geno-toxic impurities, several amines and acidic compounds.

Abstract:

Introduction

Traditionally, the process of LC/MS amenable separation method development has been time consuming as well as labor intensive task. UHPLC based method scouting system will dramatically shorten total run time comparing to the conventional system and enables to output multiple results based on the various combination of different mobile phase and multiple columns allow you to make the comprehensive investigation of analytical conditions. And also, it is well known the sensitivity of mass spectrometric detection will be varied with the type of mobile phase. In this study, the UHPLC based method scouting system coupled to single quadrupole mass spectrometer was applied to develop the LC/MS amenable method for the simultaneous analysis of geno-toxic impurities, several amines and acidic compounds.

Methods

UHPLC based method scouting system (Nexera Method Scouting System, Shimadzu Corporation, Japan) is configured by Nexera UHPLC modules. For the detection, single quadrupole mass spectrometer (LCMS-2020, Shimadzu Corporation, Japan) was used. The system can be operated at maximum pressure 100MPa and enables to automatically select up to 96 unique combinations of 8 different mobile phases as well as 6 different columns. To control the system, a dedicated control software was newly developed. This software (Method Scouting Solution, Shimadzu Corporation, Japan) allows you to visually configure the different type of columns and mobile phases. Thanks to the seamless integration with workstation software for LC/MS (LabSolutions, Shimadzu Corporation, Japan), batch queue can be created automatically.

Preliminary Data

Some geno-toxic compounds such as benzene sulfonic acid ethyl, toluene sulfonic acid butyl and toluene sulfonic acid ethyl as well as amines and acidic compounds were selected as experimental samples. Those geno-toxic compounds have low polarity and are supposed to be retained by ODS column. Therefore, Shim-Pack XR-ODSII was selected. Aqueous phase must be volatile for LCMS, 4 kinds of aqueous phase were selected as following : 1) water, 2) 0.1 % formic acid / water, 3) 0.1 % formic acid + 5 mmol/L ammonium formate water and 4) 5 mmol/L ammonium formate - water. For organic phase, acetonitrile, methanol and acetonitrile / methanol (50 / 50) were selected. Totally, 12 conditions were investigated by using Nexera Method Scouting System. In case of geno-toxic compounds, the combination of 5 mmol/L ammonium formate / water and methanol was selected. Furthermore, the gradient program was optimized using above mobile phase condition and analytical method for geno-toxic compounds were successfully developed. In case of amines, due to the high polarity, those could not be retained amply by ODS column. For amines, PFP column was selected. As the sensitivity is the primary concern for LC/MS method development, the combination of 0.1 % formic acid and methanol was selected even though there was other HPLC condition has shown better chromatographic separation. Finally, the analytical method of amines was established productively after the investigation of multiple gradient programs. In case of acidic compounds, those needed acidic condition for better chromatographic separation.

PTh-194
13:30 – 14:40

Detection of Emerging Legal Highs and Common Drugs of Abuse in Drug Seizures by Fast LC-MS/MS Method

Kheng Aik Lim[1], Yuan Yuan Cheryl Yeo[1], Peiting Zeng[2], Lijie Yu[1], Mei Ching Ong[1], Tiong Whei Angeline Yap[1]

[1]Health Sciences Authority, Singapore, [2]Shimadzu (Asia Pacific) Pte Ltd, Singapore

Keywords:
LC-MS/MS, Synthetic Cannabinoids, Synthetic Cathinones, Neutral Loss, Precursor Ion

Novel aspects:
This study is to establish the identification of legal highs via rapid LC-MS/MS scan method and their analogues via neutral loss and precursor ions scan modes.

Abstract:
One of the biggest challenges faced by forensic drug laboratories nowadays is the advent of legal highs - drugs designed to circumvent drug laws. They are usually analogues of existing controlled substances, with some structural modifications created to fall outside of legislation and to avoid identification using traditional testing methods. The analysis of legal highs can be very problematic due to the lack of drug reference standards for these new substances.

In this paper, we expand upon an existing Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) assay for controlled drugs of abuse to include about 20 new synthetic cannabinoids and cathinones that have been encountered by our laboratory or recently reported in literature. The updated assay was developed with a reduced analysis time to offer fast and selective detection of these legal highs along with current controlled drugs of interest. Drug standards of these synthetic cannabinoids and cathinones were analysed and the optimized Multiple Reaction Monitoring (MRM) for each compound was determined.

Based on the MRM data obtained as well as a study of the drugs' fragmentation patterns, we have developed screening methods for the two most common groups of legal highs : synthetic cannabinoids and cathinones. As each of these groups share a similar scaffold, the methods utilize precursor ion and neutral loss scans that allow screening for novel analogues even without the availability of drug standards. This technique is extremely useful for detecting the presence of any new legal high with no known MRM transitions or reference MS spectrum that would otherwise have been overlooked.

PTh-195

11:10 – 12:20

Untargeted detection of novel abused substances using high-resolution time-of-flight mass spectrometry with comprehensive collision-induced dissociation

Michiko Kanai[1], Kevin Siek[2], Joe Binkley[2], Jeffrey S Patrick[2], David Alonso[2]

[1]LECO Japan Corporation, Tokyo, Japan, [2]LECO Corporation, MI, USA

Keywords:
High-resolution TOFMS, Comprehensive In-source CID, Forensic analysis

Novel aspects:
Coupling comprehensive In-source CID with high-resolution TOFMS enables advanced signal processing algorithms and data interrogation tools to rapidly detect and confirm novel abused substances.

Abstract:
To accurately map the rapidly evolving landscape of chemical substance abuse requires analytical technologies that can detect targeted and untargeted analytes with equal facility. High-resolution time-of-flight mass spectrometry can offer the forensic scientist such capabilities, but in practice, extracting signals of untargeted drugs from volumes of high-resolution spectrometric data proves challenging. Coupling comprehensive collision-induced dissociation (CID) with high-resolution time-of-flight mass spectrometry enables advanced signal processing algorithms and data interrogation tools to rapidly detect and confirm novel abused substances present in urine or seized materials.

The mass spectrometric technology and the data processing strategies discussed in this presentation were used to identify a designer psychostimulant and its metabolites present in human urine, and to identify an unknown drug and an unknown metabolite present in racehorse urine. High-resolution time-of-flight technology aided the identification of structurally diagnostic fragment ions for the synthetic cannabimimetic JWH-018. Extending high-resolution time-of-flight mass spectrometry with comprehensive CID to detection of designer steroids is also discussed briefly.

PTh-196
13:30 – 14:40

Identification of triazolam, etizolam and their metabolites by liquidchromatography tandem mass spectrometry

Mayumi Matsui[1], Toshikazu Minohata[1], Noriko Shoji[2], Naohiro Kuriyama[2], Chie Yokoyama[2], Keiko Matsumoto[1], Jun Watanabe[1], Junko Iida[1]

[1]Shimadzu Corporation, Kyoto, Japan, [2]YMC Co., LTD., Komatsu, JAPAN

Keywords:
LC-MS/MS, forensic, etizolam, triazolam, metabolites

Novel aspects:
Simultaneous analysis of Triazolam, Etizolam and their metabolites usinghigh resolution hybrid silica-based UHPLC column coupled with LC-MS/MS

Abstract:
Benzodiazepines are among the most frequently prescribed drugs due to their sedative, hypnotic, anxiolytic, muscle relaxant and antiepileptic properties. Because of the high consumption of benzodiazepines worldwide, this class of drugs and their metabolites are frequently present in both clinical and forensic cases. For these reasons, the analysis of
benzodiazepines and their metabolites in biological fluids is of great interest to clinicians and forensic toxicologists. It's been hard to identify Etizolam, Triazolam, and their metabolites (alpha-Hydroxyetizolam, 8-Ethylhydroxyetizolam, alpha-Hydroxytriazolam and 4-Hydroxytriazolam) mixture because of a similar chemical structure, molecular weight and mass spectra of these drugs. Here, we report a high resolution separating method for simultaneous determination of 2 benzodiazepine drugs and their metabolites developed using LC-MS/MS.

As samples, three samples were prepared ; (A) mixture sample of standards of alpha-Hydroxytriazolam, 4-Hydroxytriazolam, triazolam and etizolam, (B) metabolized sample of triazolam and etizolam and (C) metabolized sample of blank. Then samples were loaded to LC-MS/MS system with a Nexera UHPLC system and LCMS-8030 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) . Separation occurred on YMC-Triart C18 column, 1.9um, 12nm (150 mm x 2 mm) at column temperature 40 C. Samples were eluted at flow rate 300uL/min with a binary gradient system ; the mobile phase consisted of (A) 10mM formic acid and (B) mixture of 10mM formic acid / acetonitrile (1 / 1) . LC-MS/MS with electrospray ionization (ESI) source was operated in multiple-reaction-monitoring (MRM) mode and MRM triggered automatic MS/MS data acquisition.

The analysis about drugs of abuse in clinical and forensic laboratories requires a highly specific detection for the simultaneous determination of several drugs. The method for forensic toxicology that we developed contains not only MRM transition parameters for quantitatibe analysis but analytical conditions and compounds information for MRM triggered automatic MS/MS parameters (product ion spectral data acquisition parameters based on the
MRM intensity as threshold) optimized for screening analysis. For typical hypnotics, we developed simultaneous analysis methods for 35 benzodiazepines and corresponding metabolites including etizolam, triazolam, and their known metabolites. In this experiment, 3 samples were prepared as mentioned above. Sample A was standard, sample B and C were metabolized samples using in human liver S9. First of all, sample A was acquired with the method of 6 MRM transition, which was etizolam, triazolam, and their known metabolites.
As a result, an excellent separation was obtained, and all 4 compounds were detected. Sample C (blank sample) was acquired and it resulted that no peak was found. Then, analysis of sample B found new three peaks detected, so 7 peaks were detected and all peaks, of which some had same MRM transition, were excellently separated. Two of the new three peaks were easily confirmed 8-Ethylhydroxyetizolam and alpha-Hydroxyetizolam because they were known metabolites. However, last one was difficult to be confirmed. Next, sample C was re-acquired with MRM triggered automatic MS/MS method and then product ion scan spectra of all peaks were acquired. These spectra were searched by typical hypnotics MS/MS library, so 6 peaks was able to assign with high hit score to corresponded compounds. It is expected that this method is applicable to the determination of drugs in forensic biological materials.

PTh-197

11:10 – 12:20

Determination of Prohibited Components in Hair Dyes by Solid Phase Extraction Coupled with Liquid Chromatography-Tandem Mass Spectrometry

Chung-Yu Chen, Tsai-Jung Lin, Maw-Rong Lee

National Chung Hsing University, Taichung, Taiwan

Keywords:

Hair dye, solid phase extraction, liquid chromatography, tandem mass spectrometry, prohibited component

Novel aspects:

SPE-LC-MS/MS for determination of prohibited components in hair dye.

Abstract:

In this study, solid phase extraction coupled with liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) was developed for determination of prohibited components, including 2,4-diaminodiphenylamine, 2,4-diaminoanisole, HC Yellow No.12, 2,3-naphthalenediol and 1-methoxy-2,5-diaminobenzene, in hair dye. The optimum extraction conditions were using SPEC MP 1 as extracting cartridge and 1 mL methanol containing 0.1% potassium hydroxide as eluting solvent. Under optimal conditions, the linearity of proposed method ranged from 50 to 5000 ng/mL for all analytes expect 2, 3-naphthalenediol (100-2000 ng/mL). The limit of detection and limit of quantitation were 6.7-16.8 ng/mL and 22.3-56.1 ng/mL, repsectively. The intra-day and inter-day precisions were 0.6-7.1% and 9.0-17.3%, resprctively. The feasibility of applying the proposed method to analyze the five illegal components in commercial hair dye was also examined. The results showed the method proposed is useful for determination of prohibited components in hair dye.

PTh-198
13:30 – 14:40

Doubly-Etched Microfabricated Gas Chromatography Columns for High Resolution Mass Spectrometry

Sanggoo Kim, Sung Min Lim, Sejong Yong

Korea Basic Science Institute

Keywords:
Microfabrication, Chromatography chip, High Resolution MS

Novel aspects:
Novel structure of microfabricated columns for GC/MS and HR/MS

Abstract:
Gas chromatography columns in borosilicate have been microfabricated for high resolution mass spectrometry. The microfabricated columns were made using a wet chemical etching process that created a 100-mm wide 50-mm deep semi-circular cross section channel. The photoresist SU-8 (both patterning and etching mask) was applied on a 500-mm thick 4-inch wafer which was subsequently immersed in a HF etching solution for 3~5 minutes. Using an aligner/exposure, the process was repeated on a single wafer. The three-dimensional etched profile of the microfabricated columns illustrated that they were comparable to fused-silica capillary columns. The inlets of the microfabricated columns were made using dry film patterning and sandblasting (0.5 MPa) . Anodic bonding was applied as bonding of the borosilicate wafer to a silicon wafer after the photoresists were selectively removed. At 500 °C for 2 hr and an applied
voltage of 900V, it offered high bond strength and effective annealing. The microfabricated columns were connected with a transfer line of the mass spectrometer. The detailed mass spectrometry using the microfabricated columns and high resolution GC/MS will be presented.

Poster Session

Thursday, 20th September

Event Hall

Core Time : 11:10 - 12:20 (Odd number), 13:30 - 14:40 (Even number)

Others

PTh-199

11:10 – 12:20

Coupling of Capillary Electrophoresis with Solvent Assisted Inlet Ionization-Mass Spectrometry Using a Poly(dimethylsiloxane)-based Sheath Liquid Interface

Che-Wei Wang, Guor-Rong Her

National Taiwan University, Taipei, Taiwan

Keywords:

capillary electrophoresis, mass spectrometry, poly (dimethylsiloxane) , solvent assisted inlet ionization, sheath liquid interface

Novel aspects:

This is the first attempt to couple CE with solvent assisted inlet ionization MS.

Abstract:

Solvent assisted inlet ionization (SAII) , developed recently by Trimpin *et al.*, produces mass spectra similar to electrospray ionization (ESI) . The analyte/solvent solution could be introduced into the inlet tube due to a pressure drop from atmospheric pressure to vacuum and ionized without the use of voltage or nebulizing gas. This characteristic was utilized to simplify the implement of CE/MS. In this work, a Poly (dimethylsiloxane) (PDMS) -based CE/MS interface using SAII as the ionization method was developed. This interface consists of a CE separation capillary, a sheath liquid delivery capillary, a PDMS substrate, and a sprayer. Because the CE eluents could be drawn directly into the inlet tube and ionized without the ESI process, both the sprayer coated with conductive materials and the MS inlet were grounded. Sheath liquid was used to reach the flow rate at which a stable spray was achieved. At a flow rate of 0.5- 2 μL/min, the sensitivity of CE/SAII-MS was comparable to that of low-sheath-flow CE/ESI-MS interface. Because of the use of no fragile nano-ESI tip, this interface provides the advantage of robustness and easy-to-use, and thus facilitates the operation of CE/MS analysis.

PTh-200
13:30 – 14:40

The Development of a Sheathless CE/ESI-MS interface Based on Thin Conducting Liquid Film

Ju-Li Huang, Ren-Yu Hsu, Guor-Rong Her

National Taiwan University, Taipei, Taiwan

Keywords:
Capillary electrophoresis - mass spectrometry, sheathless interface, conducting liquid film.

Novel aspects:
A simple and robust two capillary sheathless CE/MS interface was constructed.

Abstract:
A simple two column sheathless CE/MS interface was constructed using polydimethylsiloxane to fabricate a microdevice allowing facile column alignment and electrical connection. A liquid reservoir and a hole with a depth of &#65374 ; 1 mm were fabricated on the microdevice at the edge of the conducting reservoir. The ESI sprayer was inserted through the reservoir to the hole so allowing it to be aligned with the separation column. The reservoir was filled with a conductive liquid prior to inserting the ESI sprayer. Electrical connection was established through the thin liquid film formed in the space between the ESI sprayer and liquid reservoir. The interface design presented was both easy to fabricate andoperate and demonstrated good performance. The dead volume did not significantly affect operation as indicated by a demonstrated preservation of separation integrity. The repeatability and reproducibility observed using this interface ranged from 3.3 % to 8.0 % and 5.2 % to 9.6 %, respectively.

PTh-201
11:10 – 12:20

Proteome analysis with molecular simulation can reveal the TTR amyloidogenesis

Kenji Miura[1], Naoya Hatano[1], Mika Ohta[1], Hirotaka Sato[2], Aki Sugano[1], Eiichi Maeda[1], Yoshiyuki Sakaki[3], Ken-ichi Yamamura[4], Yutaka Takaoka[1]

[1]Kobe University Graduate School of Medicine, Kobe, Japan, [2]Iwate Medical University School of Dentistry, Iwate, Japan, [3]Toyohashi University of Technology, Aichi, Japan, [4]Kumamoto University, Kumamoto, Japan

Keywords:
transthyretin, hemopexin, amyloidogenesis, MD simulation

Novel aspects:
Proteome analysis with molecular simulation can reveal the TTR amyloidogenesis

Abstract:
7.2-hMet30 mice is a disease model of familial amyloidotic polyneuropathy (FAP), that consists of human amyloidogenic mutant transthyretin (TTR V30M) [1]. We previously found that 60% of this model showed amyloid depositions. In addition, nonfibrillar TTR depositions were found in 2 of 12 Amyloid-negative 7.2hMet30 mice. These results suggest that the TTR amyloidogenesis is caused not only by mutant TTR but also additional factor (s) such as etiologic molecule (s).
To find the differences between amyloid-positive and negative in 7.2-hMet30 models, we analyzed the sera of these mice models by proteome analysis. We firstly analyzed by SDS-PAGE. Secondly, in-gel digestion with trypsin was performed and the resulting protein digests were analyzed by MALDI-TOF mass spectrometry. Among differentially displayed protein bands, we found hemopexin and transferrin which were showed only in amyloid-positive mice, but not in the amyloid-negative mice. We also confirmed these molecules by Western blotting. These two proteins were not detected in the nonfibrillar-TTR deposition by immunohistochemical staining. These results suggest that the two molecules may accelerate amyloidogenesis of TTR V30M.
We then simulated the docking of native TTR or TTR V30M to hemopexin or transferrin and analyzed the binding affinity of the total four pairs. Finally, molecular dynamics (MD) simulations of the TTR-transferrin or TTR-hemopexin complex were performed to elucidate the involvement of hemopexin or transferrin in amyloidogenesis. The docking simulation showed that binding affinity of TTR V30M and each of the two proteins - hemopexin and transferrin - were both higher than that of native TTR to each protein. In addition, MD simulation analysis showed that hemopexin and transferrin may facilitate the degradation of beta-strand in TTR-hemopexin and TTR-transferrin complex. This result suggests that these molecules accelerate fibrillogenesis of TTR because preceding studies revealed that TTR amyloid is triggered by beta-strand degradation.
In conclusion, our results suggest that hemopexin and/or transferrin associate with TTR amyloidogenesis *in vivo.*

[1] Takaoka, Y., Ohta, M., Miyakawa, K., Nakamura, O., Suzuki, M., Takahashi, K., Yamamura, K., and Sakaki, Y., Cysteine 10 is a Key Residue in Amyloidogenesis of Human Transthyretin Val30Met, *The American Journal of Pathology*, 164 (1) : 337345, 2004.

Plenary Lecture

Friday, 21st September
08:00 – 08:45
Main Hall

Plenary Lecture 5: Richard M Caprioli

Chair: Dominic M Desiderio (University of Tennessee Health Science Center, USA)

PL5-0800 Molecular Imaging of Tissues by Mass Spectrometry: Looking Beyond the Microscope

08:00 – 08:45

Richard M Caprioli
Vanderbilt University, Nashville, TN, USA

Keywords:
Imaging MS

Novel aspects:
Technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns) and at high speeds.

Abstract:
Imaging MALDI MS produces molecular maps of peptides, proteins, lipids and metabolites present in intact tissue sections. It employs desorption of molecules by direct laser irradiation to map the location of specific molecules from fresh frozen and formalin fixed tissue sections without the need of target specific reagents such as antibodies. Molecular images of this nature are produced in specific m/z (mass-to-charge) values, or ranges of values, typically covering the MW range 200-100,000. We have also developed a similar approach for the analysis of targeted areas of tissues by integrating mass spectrometry and microscopy, termed histology-directed molecular analysis, whereby only selected areas of cells in the tissue are ablated and analyzed.

We have employed Imaging MS in studies of a variety of biologically and medically relevant research projects, such as developmental studies of embryo implantation is mouse and studies of disease including kidney disease, cancers, and neurodegenerative disease. Molecular signatures have been identified that are differentially expressed in diseased tissue compared to normal tissue and also in differentiating different stages of disease. These signatures typically consist of 10-20 or more different proteins ; each identified using classical proteomics methods. In addition, Imaging MS has been applied to drug targeting and metabolic studies both in specific organs and also in intact whole animal sections following drug administration.

This presentation will focus on recent technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns) and at high speeds so that a typical sample tissue (e.g., a whole mouse brain section) can be imaged in less than 10 min. Some selected examples will include studies of diabetic nephropathy, tumor bearing tissues and normal developmental processes. Other aspects of the technology, such as 3-D imaging, will also be considered. Finally, new biocomputational approaches will be discussed that deals with the high data dimensionality of Imaging MS and our implementation of 'image fusion' in terms of predictive integration of MS images with microscopy and other image modalities.

Oral Session

Friday, 21st September

Main Hall

09:00 – 11:00

Session 41: Chemistries of Trapped Ions and their Applications to Bio- logical Mass Spectrometry

Chair: Gavin E Reid (Michigan State University, USA)

S41-0900

09:00 – 09:40

[Keynote Lecture] Bioconjugation in the Gas Phase: New Chemistry for Tandem Mass Spectrometry

Scott A McLuckey
Purdue University

Keywords:
Tandem MS, ion/ion reactions, bioconjugation

Novel aspects:
Novel specific gas phase chemistries for selective modification of primary amines, quanidinium groups, and carboxylate groups.

Abstract:
The advent of ionization methods that form multiply charged ions from biomolecules has allowed for the exploration of ion/ion reactions as a new class of chemical reactions in analytical mass spectrometry. Attractive features common to all ion/ion reactions are that they are highly exothermic for virtually any anion/cation combination and that the overall reaction kinetics are independent of the chemical identities of the reactants. The ion/ion reactions of multiply charged peptide, protein, and oligonucleotide ions involving proton transfer, electron transfer, or metal ion transfer have been extensively studied. Recent results have shown that much more complex types of reaction that involve bond formation and bond cleavage can also take place during the course of an ion/ion reaction. These results demonstrate that selective chemistries can be effected between stages of mass spectrometry. Some of these reactions are those normally associated with bio-conjugation chemistries in solution.

The ability to conduct bioconjugation chemistries in the mass spectrometer enable new approaches to derivatize analyte species of interest for structural characterization. This presentation will summarize what is currently known about the underlying factors that govern the outcomes of ion/ion reactions that involve selective chemistries. Several examples will be given to demonstrate the utility of gas-phase derivatization for primary and tertiary structure determination of peptide and protein ions.

All of the data underlying the findings related in this talk have been collected in either three-dimensional quadrupole ion traps or linear ion traps operated in the presence of a background gas at 1-10 mtorr.

This presentation will summarize current understanding of the dynamics of gas-phase bio-ion/ion reactions with emphasis on the factors that determine the major observed pathways. Phenomena common to all such reactions will be described first followed by the implications for particular reaction types. Of particular emphasis in this presentation will be electron transfer, single proton transfer, multiple proton transfer is a single ion/ion encounter, metal transfer, and attachment. Essentially all of these outcomes can be dominant for a given analyte, depending upon the form of the analyte ion and the identity of the reagent. This presentation will draw on data, both old and new, that illustrate the underlying factors that make reagents ions either particularly well-suited or poorly suited for a particular analyte ion transformation of interest.

Oral Session

Friday, 21st September

Main Hall

09:00 – 11:00

Session 41: Chemistries of Trapped Ions and their Applications to Bio- logical Mass Spectrometry

Chair: Gavin E Reid (Michigan State University, USA)

S41-0940

09:40 – 10:00

Trap that Fat: Structure elucidation of ionized lipids by selective ion-molecule reactions and radical directed dissociation

Huong T Pham[1], Alan T Maccarone[1], Tony Ly[2], Adam J Trevitt[1], Larry J Campbell[3], Todd W Mitchell[1], Stephen J Blanksby[1]

[1]University of Wollongong, Wollongong, Australia, [2]University of Dundee, UK, [3]AB Sciex, Concord, Canada

Keywords:
Ion activation, Ozone induced dissociation, Radical directed dissociation, Lipidomics, Ion-trap mass spectrometry

Novel aspects:
Ozone induced dissociation and radical directed dissociation in ion trap mass spectrometers provide novel and complementary capabilities for lipid structure elucidation that are compatible with high throughput shotgun lipidomics

Abstract:
Molecular structure elucidation in the fast emerging field of lipidomics presents some unique challenges to the traditional ion activation methods employed in contemporary mass spectrometers. Notably, isomeric lipids that occur frequently in nature exhibit quite distinct biochemical and biophysical behaviour but can be difficult to differentiate by mass spectrometry alone. For example, the conjugated linoleic acid, CLA (10*E*,12*Z*) has been associated with atherosclerosis while its isomer CLA (9*Z*,11*E*) - differing only in the positions of carbon-carbon double bonds - was shown to inhibit the disease. Conventional strategies for modern lipidomics rely on electrospray ionisation (ESI) to form even-electron ions with subsequent low energy collision induced dissociation (CID) for structural characterisation. Under such conditions however, product ions arising from dissociation of carbon-carbon bonds within the hydrophobic chains of lipids are typically absent or of low abundance. As a result, ESI-CID spectra obtained from isomeric lipids are often indistinguishable particularly where the molecular structures differ in motifs such as : the positions and stereochemistry of carbon-carbon double bonds ; the positions and size of carbocyclic structures ; or positions of chain branching. In contrast simple lipids, such as fatty acid methyl esters, have been analysed by electron ionisation (EI) for decades and the resulting radical cations generated in this process lead to extensive fragmentation of the hydrocarbon chain. Unfortunately the high energies imparted by EI also lead to extensive rearrangements with a consequent loss of structural specificity and here too differentiation of isomers (of the types described above) can be ambiguous.

In this presentation we will outline two alternative activation methods that rely on the selective reaction of unsaturated lipids with ozone (ozone induced dissociation or OzID) and the generation and dissociation of radical ions generated by photolysis (radical directed dissociation or RDD) . Recent studies of the gas phase reactions of mass-selected even-electron ions with ozone on a tandem linear ion-trap mass spectrometer (QTRAP2000, AB Sciex, Concord, Canada) reveal that lipids with conjugated double bonds have a significant enhancement in reaction rate (up to 120 times) relative to non-conjugated isomers. Furthermore, ozonolysis of conjugated double bonds gives rise to unique radical dissociation pathways that are diagnostic of the presence and location of this bonding motif. The speed and selectivity of this ion-molecule chemistry is such that the triple quadrupole geometry of the QTRAP can be exploited to undertake sensitive neutral loss surveys of complex lipid extracts to search for specific isomers of conjugated linoleic acids that are commonly present at low abundance with respect to non-conjugated variants. In parallel, we have applied RDD to the study of lipids for the first time. In this approach, bifunctional molecules that contain a photo-caged radical initiator and a lipid-adducting group, such as 4-iodoaniline and 4-iodobenzoic acid, are used to form non-covalent complexes (*i.e.*, adduct ions) with a lipid during electrospray ionization. Laser irradiation (266 nm) of the mass selected complexes within a single-stage linear ion-trap mass spectrometer (LTQ, Thermo Fisher, San Jose, CA) cleaves the carbon-iodine bond to liberate a highly reactive phenyl radical. Subsequent activation of the nascent radical ions results in RDD with significant fragmentation along the hydrocarbon chain. This approach provides diagnostic product ions that are associated with the double bond position and the positions of chain-branching in phospholipids, sphingomyelins and triacylglycerides and has thus been used to differentiate isomeric lipids differing only in such motifs.

Oral Session

Friday, 21st September

Main Hall

09:00 – 11:00

Session 41: Chemistries of Trapped Ions and their Applications to Bio- logical Mass Spectrometry

Chair: Gavin E Reid (Michigan State University, USA)

S41-1000

10:00 – 10:20

Harvesting High-Level Structural (Stereo- and Anomric-) Information from Oligosaccharides with Single Sugar Resolution - A Tandem Mass Spectrometric Approach

Chiharu Konda[1], Frank A Londry[2], Brad Bendiak[3], Yu Xia[1]

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Keywords:
tandem mass spectrometry, oligosaccharide

Novel aspects:
Determining the stereochemistry and anomeric configuration of each sugar units within an oligosaccharide via multiple stage beam-type CID on a triple-quadrupole/liner ion trap instrument.

Abstract:
Carbohydrates are ubiquitous in cells. In addition to providing energy and being structural components in cell walls, they play important roles in cell-cell interactions, cell signaling, self and non-self recognition events. Understanding these roles requires that their structures be determined with confidence. Due to the structure complexity of carbohydrates, four levels of structure information need to be determined, including: 1) the stereochemistry of each sugar (subunit identity), 2) anomeric configurations, 3) linkage positions between connecting sugars, and 4) any modifications on substituents. Tandem mass spectrometry (MS^n) has been widely applied in structural analysis of carbohydrates for determining the sequence, branching, linkage type, and positions of substituents. Recently, an MS^3-CID method has been developed to pinpoint the stereochemistry and anomeric configuration of the non-reducing sugar of hexose-containing disaccharides having any of the 16 possible stereo-chemical variants. This approach was based on the distinct CID fragmentation patterns of *m/z* 221 product ions, having the structure of a non-reducing sugar glycosidically linked to a glycolaldehyde (GA), derived from CID of deprotonated disaccharides. In this presentation, we further applied this methodology to small oligosaccharides by using MS^n - CID (n= 4 and 5) to sequentially break oligosaccharide anions down to dimmers and finally perform CID on *m/z* 221 diagnostic ions. By comparing the CID patterns of *m/z* 221 ions to that of the standards (monosaccharide-GA), the identity and anomeric configuration of each sugar unit within the oligosaccharide could be determined for the first time. A series of tri-, tetra-, and penta- saccahrides were used to demonstrate the capability of this method. All experiments were performed on a modified triple quadrupole linear ion trap mass spectrometer (QTRAP 4000). This instrument allowed high efficiency bi-directional ion transfer between quadrupole arrays, where multiple-stage (two or three) beam-type CID could be enabled to produce the ladder of fragment ions within a MS^4 or MS^5 experiment.

Oral Session

Friday, 21st September — Main Hall

09:00 – 11:00

Session 41: Chemistries of Trapped Ions and their Applications to Bio- logical Mass Spectrometry

Chair: Gavin E Reid (Michigan State University, USA)

S41-1020
10:20 – 10:40

Measurement of the Peptide Ion Temperature by Using N-Acylated Dipeptide Tags as Internal Standards

Seung Koo Shin, Jongcheol Seo, Min-Soo Suh, Hye-Joo Yoon
Pohang University of Schence and Technology, Pohang, Korea

Keywords:
ion temperature measurement, N-acylated dipeptide tag, internal standard, tandem mass spectrometry, peptide fragmentation

Novel aspects:
N-acylated dipeptide tags, isobaric tags originally developed for protein quantification, enable precise measurement of the ion temperature in peptide fragmentation, regardless of the peptide sequence and the charge state.

Abstract:
Peptide fragmentations into the b- and y-type ions are useful for identification of proteins. The b ion that dissociates to the a-type ion with loss of CO has the structure of *N*-protonated oxazolone. The kinetics of this unimolecular CO-loss process allows measurement of the temperature of the oxazolone ion. Herein, we report a use of *N*-acylated dipeptide tags, isobaric tags originally developed for protein quantification, as internal standards for measurement of the ion temperature in peptide fragmentation. Amine-reactive dipeptide tags were attached to the *N*-termini of sample peptides and collision-induced dissociation of the tagged peptides yielded the b-type quantitation signal (b_S) from the tag. The b_S ion subsequently dissociated into the a_S ion upon CO-loss and the yield of a_S ion was obtained from the mass spectra. As the length of alkyl side chain on the dipeptide tag was extended from C_1 to C_8, the a_S-ion yield gradually increased for the 4-alkyl substituted oxazolone ion but decreased for the 2-alkyl substituted one. To gain insights into the unimolecular dissociation kinetics, we obtained the potential energy surface from ab initio calculations. Resulting potential energy surfaces were used to calculate the microcanonical and canonical rate constants as well as the a_S-ion yield. Arrhenius plots of canonical rate constants provided the activation energy and pre-exponential factor for the CO-loss process in the 500-900 K range. Comparison of the experimental a_S-ion yield with the theoretical one led to precise determination of the temperature of the b_S ion. Thus, the b_S-ion temperature of any peptide can be measured simply by combining kinetic parameters and the experimental a_S-ion yield, regardless of the peptide sequence and the charge state. Significantly, when the b_S-ion temperatures were nearly the same with only a few K difference, the y-type fragment patterns were almost identical to each other. Our results demonstrate a novel use of *N*-acylated dipeptide tags as internal standards to measure the b_S-ion temperature that can be a good parameter to obtain reproducible peptide fragment spectra.

Oral Session

Friday, 21st September

Main Hall

09:00 – 11:00

Session 41: Chemistries of Trapped Ions and their Applications to Bio- logical Mass Spectrometry

Chair: Gavin E Reid (Michigan State University, USA)

S41-1040 Gas phase reactions of trapped bioorganic ions

10:40 – 11:00

Gianluca GIORGI
University of Siena

Keywords:
nuclephilic additions ; ion trap ; DFT calculations

Novel aspects:
Study of nuclephilic additions to fluorinated triazine and imidazole cations that show interesting aspects of their gas phase reactivity and adds to the very few examples reported in the literature

Abstract:
The study of properties and reactivity of organic and bioorganic ions in the gas phase is an interesting challenge in several fields of research, ranging from drug design to biotechnology and nanomaterials, to optoelectronics.
Many kinds of gas phase reactions can occur in ion traps. These can involve ion-molecule, ion-ion interactions, even- or odd-electron positive or negative species, and can occur through different mechanisms, such as nucleophilic or electrophilic additions, substitutions, eliminations, etc.
In our research activity, aimed at investigating gas phase ion chemistry and reactivity of bioorganic ions, we studied different kinds of ion-molecule reactions, mainly consisting in nucleophilic additions, produced by trapped ions. Those occurring in two series of heterocyclic compounds are shown in this work.
Triazines represent a class of interesting heterocyclic compounds with applications in several fields, such as pharmaceutical chemistry, agrochemistry, and biology. A new series of fluorinate triazines have been ionized by electron ionization or electrospray thus producing radical cations, protonated or deprotonated molecules. When submitted to low-energy collision-induced dissociations, their protonated molecules show interesting elimination/hydration reactions consisting in consecutive losses of HF followed by nucleophilic additions of water that produce puzzling ions in their MS^nspectra. DFT calculations carried out on different stable species and transition states, have allowed us to elucidate sites and mechanisms of these reactions and to propose a reaction pathway.
Imidazoles are an important class of heterocycles that play several roles not only in biology, but in also emerging disciplines, such as nanotechnology and molecular engineering, due to their capability to produce nanostructured materials.
A series of diphenylimidazole derivatives protonated by ESI, show addition of water and alcohols to their acylium cations thus producing diol and carboxylic acid derivatives. The kinetics of these reactions has been studied by selecting the precursor ions, varying the activation time, and monitoring the abundance of product ions. Two different pathways yielding elimination of benzene have been also found and studied by isotopic labeling.
Collision induced decompositions involving losses of radical species from even-electron cations and anions produced by these two classes of compounds, as exceptions to the "even-electron rule " will be also presented.
DFT theoretical calculations have been carried out on different neutral and charged species by using the B 3 LYP density functional method with the triple-zeta basis set 6 -311+G (d,p) for evaluating their structure, stability and to propose reaction pathways.

Oral Session

Friday, 21st September
09:00 – 11:00
Room A

Session 42: New Developments in Instruments and Detectors

Chair: Takaya Sato (JEOL, Japan)

S42-0900
09:00 – 09:40

[Keynote Lecture] From supercomputer modeling to highest mass resolution in FT ICR

Evgeny N Nikolaev[1,2]
[1]The Institute for energy problems of chemical physics Russian Academy of Sciences, [2]The Institute of biochemical physics Russian Academy of Sciences

Keywords:
FT ICR,cell harmonization,superhigh resolution

Novel aspects:
New FT ICR detector cell disign. Superhigh mass resolution. High mass accuracy. Fine structure of peptide mass spectra. Isotopic resolution for near 200 kDa proteins at 7 Tesla

Abstract:
Since its opening by Alan Marshall and Melvin Comisarow in 1974, FT ICR Mass Spectrometry was always attracting special attention in mass spec community because of the extremely high mass resolution and high mass accuracy it demonstrated. The early attempts to explain high mass resolution were limited by analyses of two factors : mathematical - the longer is time domain, the narrower is the mass peak, and physical -collisions with residual gas molecules are reducing synchronous motion of ions and thus decreasing time domain signal longitude. The first attempts to analyze the nature of pick broadening in FT ICR spectra using supercomputer, undertaken by Nikolaev's group in 1995, have shown that there is mass resolution limitation caused by electric field distribution in FT ICR measuring cell. Electric field deviation from ideal hyperbolic distribution causes losses of ion cyclotron motion synchronization, transformation of ion clouds into comet like structures and signal degradation. This phenomenon depends only on FT ICR cell design. The other phenomena - peak coalescence, which strongly influences ion cloud behavior (initially observed experimentally) , has been reviled by supercomputer simulations based on Particle in Cell (PIC) algorithm first introduced for FT ICR simulations by Dale Mitchell and further developed by Nikolaev's group. PIC algorithm permitted to observe the new phenomenon in ion cloud behavior - absence of comet formation at large number of ions in the cloud. We called this effect "condensation ". The critical number of ions for occurrence of condensation is independent of magnetic field strength. Currently available software for simulation of ion cloud behavior in FT ICR cell during all stages of mass spectra measurement procedure includes different methods of solving Laplace and Poisson equations such as capacity matrix method for ICR cell of arbitrary geometry. Understanding of behavior of ion ensembles inside FT ICR cell gives rise to the new ideas of cell designs. Among them is the idea of dynamic harmonization of the cell. The recently introduced novel FT-ICR cell based on a Penning ion trap with specially shaped excitation and detection electrodes prevents distortion of ion cyclotron motion phases (normally caused by non-ideal electric trapping fields) by averaging the trapping DC electric field during the ion motion in the ICR cell. This effect results in mass resolving powers which exceed any values obtained up to now. The performance of the novel FT-ICR cell with shaped electrodes has been characterized by isotopically resolved mass spectra of proteins close to 200kDa as well as fine structure of resolved isotopic peaks of peptides and small proteins. Accordingly, detection times of 5 minutes resulting in resolving power close to 40,000,000 have been reached for reserpine at m/z 609 at a magnetic field of only 7 Tesla. Furthermore, fine structures of resolved 13Cn isotopic cluster groups could be measured for molecular masses up to 5.7 kDa (insulin) with resolving power of 4,000,000 at 7 Tesla. Based on resolved fine structure patterns atomic compositions can be directly determined using a new developed algorithm for fine structure processing. Extremely high mass accuracy below 100 ppb (RMS mass error) could be achieved in complex mixtures. As example for high mass species, proteins and multimers of proteins could be measured reaching masses up to 186 kDa (enolase tetramer) with isotopic resolution. For instance, at 7 Tesla resolving power of 800,000 was achieved for enolase dimer (96kDa) and 500,000 for molecular masses above 100 kDa. Experimental data indicate that there is practically no limit for the resolving power of this ICR cell except by collisional damping in the ultrahigh vacuum chamber.
Acknowledgements : Ivan Boldin,Anton Grigor'ev,Oleg Harybin,Gleb Vladimirov,Igor Popov,and Bruker Daltinics-Roland Jertz,Jens Fuchser,Claudia Kriete,Matthias Witt,Jochen Friedrich,Gokhan Baykut

Oral Session

Friday, 21st September

Room A

09:00 – 11:00

Session 42: New Developments in Instruments and Detectors

Chair: Takaya Sato (JEOL, Japan)

S42-0940

09:40 – 10:00

A New magnetic TOF Detector with 1.5 ns Pulse Width and Very High Dynamic Range

Dick Stresau, Yair Benari, Kevin Hunter

ETP Electron Multipliers, Melrose Park (Sydney), Australia

Keywords:
TOF detector, magnetic detector, electron-multiplier

Novel aspects:
A new TOF detector delivers 5 volt linear pulses with 1.5 ns pulse width, and floatable to 15 kV.

Abstract:
During the past several years major advances in operational life and dynamic range have been achieved for very high performance magnetic TOF detectors (<0.5 ns pulse width) , as reported by this group at previous conferences. The concepts employed to achieve these results have now been applied to detectors which are smaller and have been designed for use in instruments with less demanding timing requirements. The result is a detector with a 1.5 ns pulse width (for multiple ions) which exhibits exceptional dynamic range and operational life.
Simulations indicate that the multiple ion pulse width of the new detector includes a ~350 ps contribution from jitter across its 17.5 x 25mm aperture. When the aperture is masked to 12 x 25 mm, this jitter is reduced to ~150 ps. For this measurement jitter is defined as the contribution to the detector's pulse width resulting from variations in transit times from different portions of the detector's input aperture.
The new detector has been designed to have nearly the same form factor as a widely used discrete dynode TOF detector, enabling a simple replacement which will result in a significant performance boost.
As well as a standard non-floating detector, a version is being developed with built-in electrical isolation that allows the detector to be floated up to 15kV while still providing a ground-referenced output signal.
Pulse dynamic range is the common term used to describe a TOF detector's linearity. This refers to the ratio of largest linear pulse to smallest measurable pulse in a single shot spectrum. Because the level of the smallest measurable pulse is determined by the details of a specific TOF system, the most meaningful measurement for a detector will be the level of the maximum linear pulse delivered by the detector (generally expressed as millivolts (mV) into a 50 ohm load) . Expressed in these terms, the new detector's linear pulse height limit exceeds 5 volts (5000 mV into 50 ohms) .

An orthogonal TOF system (with an electron impact ion source) and a 12 bit, 2 Gsample/second digitizer will be utilized to help evaluate the new detector. The digitizer has ~1000 : 1 useful dynamic range for a single shot spectrum. In reality a single ion should be digitized with an average pulse height of ~ 10 LSB's (least significant bits) to efficiently detect all incident ions. (Single ion collection efficiency is critical in dynamic range/ linearity studies , as multiple ion events will always have near 100% efficiency, so any single ion inefficiencies will inappropriately appear to be a non-linearity.) Therefore this system is capable of accurately collecting single shot spectra with an ion abundance ratio (dynamic range) of ~100 : 1. In previous work we have made use of a much smaller digitizing range and merely looked for distortions in abundance ratios (typically ~10 : 1) at high input ion flux levels to establish the detector's dynamic range capability. The 12 bit digitizer enables collection of more realistic spectra and its 100 : 1 dynamic range enables the use of carbon isotopes which provide a convenient series of markers for this study.

Work is progressing towards generating spectra demonstrating accurate abundance ratio measurements of 100,000+ : 1 accumulated within practical averaging times (minutes) for multiple spectra. This data will be shown in the presentation along with diagrams detailing the detector's design and operational characteristics.

Oral Session

Friday, 21st September

Room A

09:00 – 11:00

Session 42: New Developments in Instruments and Detectors

Chair: Takaya Sato (JEOL, Japan)

S42-1000 Novel Compact Double Focusing Mass-Analyzer for Biochemical and Elemental Analysis.

10:00 – 10:20

Viacheslav D Sachenko, L N Gall, R N Gall
Institute for analytical Instrumentation of RAS

Keywords:
double focusing mass-analyzer, mass resolution, line of focuses, spectrographic mode

Novel aspects:
a novel ion optical scheme of the double focusing mass-analyzer for biochemical and elemental analysis, and SIMS devices

Abstract:
Static mass-analyzers (MA) loose their positions during last 20 years, and are being replaced by dynamic MA : by quadrupole mass filters for tasks requiring a low resolution, by TOF's for a wide mass range, and by ion traps for high sensitivity. The main reasons are traditional drawbacks of static MA : low scanning speed, limited mass range, large size and heavy weight. However, dynamic mass analyzers have brought their own drawbacks, and the main is the bad quality of mass-spectrometric line. For example, a modern multipass TOF showing resolution higher than 100,000 at half-height will show less than 5000 at 5 %, and will loose addiyionally about 3 orders of magnitude at 0.5 % (!) .This makes practically impossible to register minor peaks located near the major ones, and necessary to use a sample pre-separation. However, a Nier-Jones static MA shows the half-height resolution nearly the same magnitude as the best TOF, but has a very good line quality characterized by the isotopic sensitivity of 10- 6 - 10-7, the resolution remains high down to the lowest intensity levels. A lot of native mixtures can be analyzed for ecological, elemental and biochemical purposes without chromatographic pre-separation as minor peaks are well defined near the major ones.

The spectrometric mode, i.e. mass analysis in scanning mode, is available for any MA. On the contrary, the spectrographic mode having the highest theoretical duty time can be realized only by a static MA when a mass-spectrum or its part is recorded simultaneously by a position-sensitive detector or a number of collectors. Up to now, this mode was used only for isotopic and elemental analysis, and the MA of *Mattauch-Herzog type* were ideal for that as they do not require scanning. Unique features of these MA result in their long life (being proposed by authors in 1934, this solution is still in use!) . The most attractive feature of their optics is the direct line of focuses with a double focusing for all ion masses. This feature allows to register simultaneously a wide mass-spectrum of ions. However, the MA of Mattauch-Herzog type have no opportunity to filter the ion beam in energy. This limits their resolution and makes these mass-analyzers ineffective with ESI and ICP ion sources, and for biochemical applications.

We present a novel scheme of static MA which combines the best features of both known double focusing ion-optical schemes, and possesses additional advantages. The new optics has the direct line of focuses by double focusing for all masses, and has a possibility to filter the ion beam in energy, and to make fast electrical scanning of the ion beam without interfering with operation of ion source. On the contrary to the Mattauch-Herzog scheme having S-shaped geometry, the new optics is C-shaped, and electrostatic lenses are added with the aim to compensate the energy dispersion coefficient, and to provide a device adjustment. The new MA has been simulated and optimized within geometrical and chromatic 2-nd order aberrations. It shows the theoretical resolution magnitude about 3500 at 5 % of the peak height for the mass range 12-1800, energy spread of 40 eV, and maximal radius 200 mm. The permanent magnet weight does not exceed 45 kg, the MA dimensions are 600x500 mm. This allows to combine the new MA with ESI or ICP ion source without a cooling cell and additional pumping stage, and to register the near-located minor and major peaks.

The novel ion-optical scheme of the double focusing mass-analyzer suits ideally for SIMS devices too.

The authors dedicate this report to the 100-anniversary of R. Herzog and A.O. Nier, made an outstanding contribution to the theory and practice of high precision mass-spectrometry.

Oral Session

Friday, 21st September
09:00 – 11:00
Room A

Session 42: New Developments in Instruments and Detectors

Chair: Takaya Sato (JEOL, Japan)

S42-1020
10:20 – 10:40

Penning traps and ICR MS in physics research: From highly-charged atomic ions to metal-clusters to short-lived atomic nuclei

Lutz Schweikhard
University of Greifswald, Greifswald, Germany

Keywords:
Penning trap, ICR, multi-reflection time-of flight MS, polyanionic metal clusters, short-lived nuclei

Novel aspects:
Several MS techniques recently introduced : implementation of MR-ToF mass separator to Penning/Paul-trap system (with new focusing) , switching of trapping voltage for increased electron attachment, SIMCO excitation, ... and corresponding results

Abstract:
Penning-trap ion storage and ion-cyclotron-resonance mass spectrometry are applied in a wide range of investigations in physics research. These methods are used for

(a) the containment of ions,
(b) the selection of particles with respect to their mass-over-charge ratio,
(c) the analysis of reactions (CID, photodissociation, electron-impact ionization and electron attachment) as well as
(d) the high-accuracy mass determination of exotic nuclear species.

The presentation will give recent examples of new techniques and applications. In particular, the following aspects will be addressed based on developments at ClusterTrap [1] , ISOLTRAP [2] and SHIPTRAP [3] as well as the Berlin-EBIT [4] recently re-installed at Greifswald :

(a) Delicate particles such as atomic ions in very high charge states can only survive if they are in no contact with any other materials. They are produced in electron beam ion sources or traps (EBIS/EBIT) which use some of the properties of "standard " ion-cyclotron-resonance devices.

(b, c) Penning traps are valuable tools in metal-cluster research. They allow a selection of the cluster sizes such that the cluster properties can be determined for one size at a time. Multiple selection, reaction and analysis steps may be applied. Furthermore, simultaneous storage of several species as different as very heavy cluster anions and electrons is possible and their reactions such as the production of multiply negatively charged clusters can be followed as a function of time. Recently, electron attachment up to a charge state of z = - 5 has been achieved [5] by changing the trapping potential during the storage period [6] .

(c, d) By measuring the cyclotron frequency of ions with even very short-lived atomic nuclei (i.e. in the sub-second range) the nuclear masses and thus their nuclear binding energies can be determined with very high accuracy. This includes nuclides that show exotic decay modes (proton decay or pairs of nuclides between which neutrino-less double-beta decays may occur) and isotopes of very heavy elements (such as nobelium [7] and lawrencium [8] , Z= 102 and 103, respectively) .

To address more and more exotic particles, ever new techniques are developed. For example, a multi-reflection ToF MS has recently been added to the ISOLTRAP setup, complementing the already existing three-story combination of one linear Paul trap and two Penning traps to a system of in total four ion traps, each with dedicated functions. In addition, new methods of ion injection and ejection as well as time focusing have been developed with this MR-ToF MS [9] and for the Penning traps a new excitation mode has been introduced (the simultaneous application of both dipolar and quadrupolar rf fields [10]) .

As much as the research areas vary as much do the techniques that are thought of to answer the corresponding challenges. As outlined above the presentation will give specific examples of very recent developments and results.

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[3] S. Rahaman et al., Int. J. Mass Spectrom. 251, 146 (2006) , DOI : 10.1016/j.ijms.2006.01.049
[4] C. Biedermann et al., Physica Scripta T73, 360 (1997) , DOI : 10.1088/0031-8949/1997/T73/118
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S42-1040
10:40 – 11:00

A multi-reflection time of flight mass spectrograph for short-lived nuclei and heavy molecules

Peter Schury[1], Michiharu Wada[2], Yuuta Ito[1,2], Sarah Naimi[2], Hermann Wollnik[3], Tetsu Sonoda[2], Sousuke Nakamura[1,2]

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Keywords:
Time of Flight Mass Spectrometry, Nuclear Masses, Trans-Uranium Nuclei, Biomolecules

Novel aspects:
A mass spectrograph capable of quickly achieving high-resolution with high-accuracy. It is efficient and able operate at in a high dynamic range of ion intensity.

Abstract:
We present the status of a new multi-reflection time of flight mass spectrograph (MRTOF-MS). The spectrograph comprises a pair of electrostatic ion mirrors connected by a field-free drift tube. Within the spectrograph ion bunches reflect between the mirrors for an extended flight path of as much as 1 km or more. By properly tuning the electric potential within the mirrors, it is possible to compensate for the initial energy spread of the ion pulse such that the time of flight becomes independent of the initial energy of the ion. We have achieved mass resolving powers of more than 100,000.
Using techniques such as Penning trap mass spectrometry (PTMS), it is possible to achieve mass resolving powers far exceeding that which can be achieved by the MRTOF-MS. However, the MRTOF-MS overcomes certain drawbacks of such a technique. In particular, it is especially well-suited for application to short-lived ($t_{1/2}$< 100 ms) and heavy (A/q > 100) ion species. This is because the observation time required for a given resolving power scales with the square root of the mass, as opposed to scaling linearly with the mass as in PTMS. As a result, while MRTOF-MS cannot compete with PTMS in maximum achievable resolving power, it is capable of achieving respectable resolving powers (R_m> 100,000) much more quickly. For heavier species, it is a factor of ten or more faster.
Additionally, the measurements are not affected by large amounts of contaminants. On the contrary, the MRTOF-MS exhibits no systematic shifts for even large amounts of simultaneous contaminants, it in fact benefits by using such contaminants as simultaneous reference measurements. In this way, the device is a true spectrograph, capable of simultaneously analyzing many species.
Fast mass measurements are particularly useful for short-lived radioactive nuclei such as are of interest e.g. for nucleosynthesis via rapid neutron capture (r-process) in stellar supernovae. Such species tend to be both heavy (A>100) and short-lived ($t_{1/2}$< 100 ms). In such cases nature imposes a maximum observation time by way of the short half-lives, creating an imperative for speedy measurements such as is achievable with the MRTOF-MS. In such cases, the contaminant tolerance is also of great value, as time need not be spent on purification.
It is similarly well suited for measuring trans-uranium nuclei. Such nuclei are produced at incredibly low rates via heavy ion nuclear fusion reactions. They are rather heavy (A > 250) and in many cases have half-lives of only a few milliseconds. These qualities are all well aligned with the characteristics of the MRTOF-MS. A planned campaign to measure such exotic nuclei is briefly discussed.
Finally, since the achievable resolving power does not diminish for heavier species, the MRTOF-MS may also be of interest for precise identification of very heavy (A/q > 1,000) molecules. The fast measurement cycle combined with the high resolving power may make it ideal as a high-throughput spectrometer for biomolecules. Results demonstrating the high resolving power for such very heavy molecules will be presented.

Oral Session

Friday, 21st September
09:00 – 11:00
Room B-1

Session 43: Novel Proteomics Methodologies

Chair: Yasushi Ishihama (Kyoto University, Japan)

S43-0900
09:00 – 09:40

[Keynote Lecture] Shifting Proteomics from a Hypothesis Generating Workflow to a Hypothesis Testing Workflow

Michael J MacCoss[1], Jarrett Egertson[1], Michael Bereman[1], Richard Johnson[1], Christine C Wu[2], Brendan MacLean[1]

[1]University of Washington, [2]University of Pittsburgh

Keywords:
proteomics, data independent acquisition, Skyline, software, quantitation

Novel aspects:
Development of data independent acquisition strategies for quantitative proteomics.

Abstract:
Proteomics technology has improved dramatically over the last decade. The technology developments have largely been directed around instrument hardware, where instruments have been developed that scan faster, are more sensitive, and have greater mass measurement accuracy. However, the basic workflow has remained largely unchanged -- mass spectrometers are directed toward the acquisition of tandem mass spectra on the most abundant molecular species eluting from a chromatography column. More recently, efforts have been focused on the acquisition of mass spectrometry data on target peptides of interest. With improvements in instrument hardware and instrument control software, the practical experimental difference between a targeted and discovery proteomics is beginning to become blurred. These analyses are a significant change from the traditional proteomics workflow and have required the development of novel computational strategies to analyze, visualize, and interpret these data. We will present work illustrating our efforts in the development of targeted proteomics and provide a vision for challenges that still need to be overcome before these analyses become routine and replace more traditional discover proteomics methodology.

Oral Session

Friday, 21st September

Room B-1

09:00 – 11:00

Session 43: Novel Proteomics Methodologies

Chair: Yasushi Ishihama (Kyoto University, Japan)

S43-0940

09:40 – 10:00

Profiling of Human Kinome Using In Vitro Kinase Assay in Combination with Quantitative Phosphoproteomics

Naoyuki Sugiyama[1], Masaki Gouda[2], Haruna Imamura[3], Masaru Tomita[1], Yasushi Ishihama[1,3]

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Keywords:
proteomics, signal transduction, protein kinase, phosphorylation, LC-MS

Novel aspects:
Novel kinome tree based on substrate specificities was generated and enabled deep analysis of phosphoproteome data.

Abstract:
Reversible protein phosphorylation regulated by protein kinases and phosphatases is a key event for signal transduction in eukaryote cells. Recently, large-scale phosphoproteomics based on LC-MS and phosphopeptide-selective enrichment has become a powerful tool to reveal the signaling mechanism. However, it still remains difficult to map all phosphoproteome data to known signaling pathways due to the incomplete data for the kinase-substrate relationship. In this study, we profiled 385 recombinant human kinases, including 21 mutants and 10 lipid kinases, by using *in vitro* kinase assay combined with quantitative phosphoproteomics approaches.

HeLa cell lysates were pretreated with alkaline phosphatase, and then reacted with or without individual recombinant protein kinase. The reaction mixtures were subjected to reductive alkylation followed by in-solution digestion with trypsin. After stable isotope dimethyl labeling using formaldehyde, phosphopeptides were enriched with hydroxy acid-modified metal oxide chromatography (HAMMOC) using titania. The phosphopeptide fractions were analyzed with LC-MS/MS, and then the phophorylated sites by kinases and the phosphorylation motifs were determined using relative peak areas with respect to those of control sample as criteria.

As a result, 175,574 kinase-substrate relationships were obtained from the *in vitro* assay using HeLa cell lysate as the substrate source. Using the kinase-substrate combination data, we extracted a total of 1,525 phosphorylation motifs targeted by 303 kinases and classified the kinases to generate a novel phylogenic kinome tree from the *in vitro* substrates specificities instead of the traditional tree based on the sequence similarity between their kinase domains. Finally, two approaches based on *in vitro* substrates and phosphorylation motifs were developed to predict kinases which are activated or inactivated by particular stimuli. Using phosphoproteome data obtained from epidermal growth factor (EGF) -stimulated cancer cells, we successfully predict kinases belonging to EGF receptor pathways by both approaches. These novel kinome profiling using *in vitro* substrates, phosphorylation motifs and phylogenic tree provides invaluable information to unveil the entire map of cellular signaling pathways.

Oral Session

Friday, 21st September
09:00 – 11:00
Room B-1

Session 43: Novel Proteomics Methodologies

Chair: Yasushi Ishihama (Kyoto University, Japan)

S43-1000
10:00 – 10:20

In-depth analysis of human proteome by High Resolution Isoelectric Focusing (HiRIEF)-LC-MS/MS

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Keywords:
HiRIEF, meter-long monolithic capillary column, quantitative proteomics, in-depth analysis

Novel aspects:
High-resolution isoelectric focusing (HiRIEF) approach successfully increases the coverage of proteome. This method provides greater possibilities for in-depth analysis of complicated biological samples, such as clinical materials.

Abstract:
Mass spectrometry (MS) based proteomics can make a valuable contribution in clinical research by providing phenotypic information. Since clinical materials are limited in amount and diverse, highly sensitive and high resolution analysis is appreciated. To gain higher coverage of proteome, currently two distinct approaches are used ; reduce of complexity of biological samples by pre-fractionation or enrichment, or usage of high-resolution chromatography to separate complicated samples without pre-fractionation. In clinical proteomics research, it is vital to employ an approach that allows an adequate analysis of proteome using small amount of materials. Such methods can be applied to small number of cells such as circulating tumors cells (CTC) in blood. Proteome analysis of CTC is of interest in diagnosis and cancer progression. Since sample loss is considerable in small amount of materials, high-resolution chromatography becomes the preferable to pre-fractionation.
Here, we will show results comparing two distinct approaches to analyze human cellular proteome : HiRIEF-LC-MS/MS and the meter-long monolithic capillary column.
[Method]
Quantitative proteomics by HiRIEF-LC-MS/MS[1,2]:
MCF-7 cells (Human breast cancer cell lines) were stimulated with endogenous ligands or vehicle, then lysed by Urea-based lysis buffer. Following tryptic digestion, peptide samples were labeled by iTRAQ, and dissolved in rehydration solution (8 M Urea) . IPG strip (pI 3.7-4.9, 24 cm, linear gradient) was re-swelled with 8 M urea with 1 % IPG phamalyte (pH 2.5-5.0, GE Health care) overnight. Peptide mixture sample was focused and passively eluted into 72 fractions. Each fraction was analyzed on Nano-LC-MS/MS (LTQ-Orbitrap Velos) with 15 cm long C18 Pico frit column (100 µm internal diameter, 5 µm bead size, Nikkyo Technos Co., Tokyo, Japan) .
The aliquot of labeled samples were analyzed by Nano-LC-MS/MS equipped with 2 m-long monolithic capillary column MonoCap® C18 High Resolution 2000 (100 µm internal diameter, GL-Sciences, Japan)
All MS/MS data were searched by MASCOT with Percolator (FDR 1 %) against SWISSPROT human database. Quantitation of iTRAQ reporter ions was conducted by Proteome Discoverer (version 1.3) . The relative abundances between the sample sets were calculated by normalizing each peptide signal to the corresponding pooled internal standard.
During the session, we will also discuss about proteome analysis from small amount of cells ("Cent Cells ") by 2 m-long monolithic capillary column .

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Oral Session

Friday, 21st September

Room B-1

09:00 – 11:00

Session 43: Novel Proteomics Methodologies

Chair: Yasushi Ishihama (Kyoto University, Japan)

S43-1020

10:20 – 10:40

Enrichment of plasma membrane proteins using cationic nanoparticle pellicles

Waeowalee Choksawangkarn[1], Sung-Kyoung Kim[1], Joe Cannon[1], Nathan Edwards[2], Sang Bok Lee[1,3], Catherine Fenselau[1]

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Keywords:

"plasma membrane proteins", "pellicle enrichment", "nanoparticles", "spectral counting"

Novel aspects:

Various cationic nanoparticles with different density, size, shape, and surface modification have been evaluated for efficient enrichment of plasma membrane proteins using the pellicle technique.

Abstract:

Unbiased mass-spectrometry based proteomic profiling of plasma membrane (PM) proteins reveals biological processes involved in communication and signaling between cells and/or environment. Understanding qualitative and quantitative changes in expressed PM proteins at certain conditions, e.g., disease state, is a key for biomarker discovery and therapeutic development. The identification of PM proteins is made difficult by their low abundance, high hydrophobicity, and fluid population. We cope with an underrepresentation in LC-MS/MS analysis by enriching PM proteins using cationic nanoparticle pellicles and optimizing the proteomic workflow for analysis of highly hydrophobic proteins. The cationic nanoparticles increase the density of plasma membrane sheets and thus enhance separation from other lysed cellular components by centrifugation. We have synthesized silica, iron oxide, and gold nanoparticles and nanowires, followed by positive charge modification with Al_2O_3, aminopropyl triethoxysilane, or aminopropyl thiol. Effects of shape, density, size, and surface modification of the nanoparticles on PM enrichment efficiency have been investigated using RPMI 8226 multiple myeloma cells grown in suspension. We have confirmed the attachment of nanoparticles/nanowires to the cell surface by SEM and EDX analysis. Three workflows have been compared that allow tryptic digestion of PM proteins solubilized in SDS prior to LC-MS/MS analysis of tryptic peptides and their parent proteins (*J. Proteome Res.*, 2012, DOI：10.1021/pr300188b). Using an optimized workflow, the enrichment efficiency of each type of nanoparticle/nanowire has been compared, based on fractions of the proteins assigned to the plasmamembrane/transmembrane/cell surface categories in the UniProt KnowledgeBase. In addition, quantitative comparison of PM proteins and PM markers has been assessed based on spectral counting. Overall, we have identified 1,415 PM proteins (based on one or more peptides), which represents about 35% of the human plasma membrane proteome listed in UniProt.

S43-1040
10:40 – 11:00

Informatics-Assisted Label-free EDC-MRM Quantitation for Targeted Signaling Pathway Analysis

Yi-Ting Wang[2,3], Chia-Feng Tsai[1,4], Pei-Yi Lin[1], Hsin-Yi Wu[1], Szu-Hua Pan[6], Sung-Liang Yu[5], Pan-Chyr Yang[3,7], Yu-Ju Chen[1,2,4]

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Keywords:
Lung cancer, phosphoproteomics, MRM, label-free, pH/acid control IMAC

Novel aspects:
A rapid and sensitive label-free quantitation strategy for high throughout over-activated pathway discovery and verification

Abstract:
Despite the promise of quantitative phosphoproteomics, analytical challenges still remain for its utility in clinical application due to its low throughput in discovery and verification stages. Due to the complexity of proteome and incompatible low acquisition speed of MS, additional time-consuming fractionation step is a critical prerequisite to enable profiling protein at low abundant levels. Furthermore, site specific verification for protein phosphorylation is most limiting bottleneck because of the lack of availability of good antibody required by conventional immunoassays.

Based on alternative long and short LC-MS/MS for peptide reference database and fast acquisition, respectively, here, we present a high speed label-free quantitation strategy for personalized phosphoproteomic profiling and verification of targeted signaling pathways in human tissues. The strategy utilizes long LC gradient to construct a reference database of protein identification, which will serve as a peptide positioning system for cross-assignment and quantitation of data obtained from short LC gradient. On the pilot study of Raji B cell, nearly 100 % of the identified peptides in long (210-min) LC-MS/MS can be informatically matched to peptides that were not selected for MS acquisition in short (40-min) LC-MS/MS based on the information of peptide sequence, m/z and aligned elution time. After elution time alignment and cross-assignment, a total of 5678 peptides were quantified in 40-min LC-MS/MS runs (SD < 0.3) in biological replicate experiments (by Q-TOF MS) . High linearity on quantitation working curve was achieved (r^2=0.991) over 200-fold dynamic range using a standard phosphoprotein β-casein. At proteome scale, high quantitative accuracy ($\log_2$ ratio= 0 ±0.27) and precision (CV=13.6%) were routinely obtained in 40-min LC-MS/MS. Compared to the precursor ion-based quantitation strategy, the high speed EDC-MRM^{HR} strategy increased 10-fold sensitivity of fragment ion detection and demonstrated superior quantitation linearity, high quantitation accuracy (<20%) and precision (<10%) . On the pilot analysis of 12 pairs of cancerous and adjacent normal human lung cancer tissues, the high speed quantitation approach first identified the constituently over-activated signaling pathways. Furthermore, the high speed EDC-MRM^{HR} was applied to verify the targeted singling pathway on Iressa resistant lung cancer cell. This strategy holds promise to translate the discovery of in vitro models to clinical specimens for analysis of targeted signaling pathway unique to a specific subtype of cancer and drug responsive targets.

Oral Session

Friday, 21st September
09:00 – 11:00
Room D

Session 44: Ambient Ionization

Chair: Jentaie Shiea (National Sun Yat-Sen University, Taiwan)

S44-0900
09:00 – 09:40

[Keynote Lecture] Ambient desorption/ionization mass spectrometry for minimal samples

Kenzo Hiraoka[1], Mridul K Mandal[1], Lee C Chen[1], Hideki Fujii[2], Kentaro Yoshimura[2], Sen Takeda[2]
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Keywords:
DBDI, Leidenfrost, PESI, sheath-flow PESI

Novel aspects:
New desorption/ionization methods have been developed for minimal gas, liquid, and solid samples.

Abstract:
[Introduction] : Ambient mass spectrometry is growing very rapidly as reviewed by Fernández et al. (Anal. Chem. 2011, 83, 4508) . In this paper, ambient desorption/ionization methods for minimal gas, liquid and solid samples developed in our laboratory will be presented. The sample amounts should be as small as possible for valuable small samples and also for least contamination of the ion source. For this purpose, we made efforts to develop high-sensitive ionization methods that need samples with minimal amounts. Desorption methods for non-volatile samples will also be presented.
[Low-pressure helium dielectric barrier discharge ionization (He DBDI)] : This was designed for high-sensitive detection of vapor samples. He DBDI detected 25 pg of desorbed HMTD (explosive) . LOD of H_2O_2 in air was 800 ppt. H_2O_2 leaking from aqueous solution of H_2O_2 (30%) contained in a plastic bottle with tightly sealed cap could be detected. This method, however, is difficult to apply to nonvolatile samples. In order to circumvent this problem, we developed desorption methods based on the Leidenfrost phenomenon and flash heating by using a hot filament.
[Leidenfrost desorption/ionization] : A pencil size cartridge heater at temperatures in the range of 250-350 °C was used. 20 mL liquid solution was dropped on the heater. The liquid floated on the heater for 10s seconds due to the Leidenfrost phenomenon. At the moment of total evaporation of liquid droplet, non-volatile molecules being concentrated in the tiny droplets were desorbed instantly induced by the last-stage explosive solvent evaporation. The vaporized samples were ionized by He DBD, dc corona, or electrospray. The LODs of cocaine, codeine, morphine, and cyclosporin in methanol solutions were found to be ppb or sub-ppb levels.
[Flash heating/desorption] : For the analysis of organic solids such as synthetic polymers, flash-heating desorption method was developed. A stainless steel (0.4 mm o.d.) filament at 100-800 °C moved up and down along the vertical axis. At the lowest position, the filament slightly touched the sample surface (~10s mm in depth) . The pyrolyzed sample vapor was ionized by He DBD. Due to the instant heating of subsurface, secondary thermal decomposition reactions should be suppressed. In fact, the protonated monomer units of polylactic acids up to 10 mer were observed.
[Probe electrospray ionization (PESI)] : To mimic FD, PESI was developed. It used a metal needle with the tip diameter of < 1 mm. The needle was driven up and down by using a linear actuator. At the lowest position, the needle captured about ~1 pL liquid samples. At the highest position, a high voltage was applied to generate electrospray. By PESI, sequential electrospray of components in the order of their surface activities was realized and thus suppression effect inevitable for capillary-based electrospray was minimized. PESI was successfully applied to the cancer diagnosis.
[Sheath-flow PESI] : PESI is applicable only to wet samples. To apply PESI to dry samples, sheath-flow PESI was developed. By using a coaxial glass or plastic capillary with a fine metal needle through it, the solvent liquid was supplied to the tip of the needle that acted as a PESI probe. The liquid flowing out of a capillary extracted the components on the sample surfaces. By scanning a US dollar bill, cocaine was detected near the edge of the bill.

Oral Session

Friday, 21st September

Room D

09:00 – 11:00

Session 44: Ambient Ionization

Chair: Jentaie Shiea (National Sun Yat-Sen University, Taiwan)

S44-0940

09:40 – 10:00

Advances in Ambient Ionization and Imaging using DART and Microplasma Ion Sources

Facundo M Fernandez[1], Joel Keelor[1], Rachel Bennett[1], Prabha Dwivedi[1], Joshua Symonds[2], Reuben Gann[1], Thomas Orlando[1,2]

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Keywords:
ambient ionization, direct analysis, DART, microplasmas, imaging

Novel aspects:
Novel applications and instrumentations for plasma and microplasma ion generation techniques are discussed

Abstract:
Ambient or "open air " surface sampling techniques are a group of ion generation approaches that can be readily coupled to mass spectrometric detectors for MS analysis of target compounds. These technologies have been coupled to a variety of mass spectrometers equipped with atmospheric pressure interfaces with only minor modifications, enabling unknown identification via fragmentation pattern matching to databases, elemental formula determination via accurate mass measurements, multi-analyte quantitation, spatially-resolved measurements, and selective ionization enhancement for target compounds of interest. Ambient MS sampling/ionization techniques such as Direct Analysis in Real Time (DART) and Desorption Electrospray Ionization (DESI) have grown in popularity because they enable the sampling of analyte under atmospheric pressure conditions from both liquid and solid phases remotely from the mass analyzer, have been used to investigate objects or surface features of a wide range of shapes, sizes and textures, can perform qualitative or quantitative analysis with no or minimal sample preparation such as dissolution, grinding, extraction or pre-concentration, and can conduct all these operations in real time with high sensitivity and minimal unwanted ion fragmentation.
Plasma-based ambient sampling techniques such as DART, FAPA, LTP, DBDI, DCBI and DAPCI involve the generation of a DC or RF electrical discharge between a pair of electrodes in contact with a flowing support gas such as N_2 or He, generating a constrained flux of ions, radicals, excited state neutrals, and electrons which trigger the ionization process. Some or all of these plasma species can be directed towards the surface being sampled, inducing desorption and ionization in a single step. Optional resistive heating of the support gas, can further enhance desorption of neutrals which are then ionized by plasma species or species derived from those. Plasma-based ambient MS instrumentation tends to be fairly simple and rugged, and can be coupled to a variety of mass spectrometers, including quadrupole ion traps, linear ion traps, and quadrupole time-of-flight, providing both MS/MS and/or accurate mass capabilities. Plasma source mass spectra tend to be relatively simple, as most of the time the analytes are ionized as one or two adduct types, simplifying peak assignment in the case of unknowns. Their applicability is generally limited to molecules below 1 KDa.
DART uses a point-to-plane atmospheric pressure glow discharge to generate metastable species in a chamber that is physically separated from the ionization region. The discharge support gas, containing metastables, is heated and directed through a grid electrode that filters ions and electrons to mitigate ion-ion and ion-electron recombination of species generated within the DART ionization source. DART can be used to sample gases, liquids and solids. In laboratory settings, gases are directly injected into the ionization region following the grid electrode, whereas liquids are generally sampled by dipping a glass capillary in them and placing it in the ionization region. Solids can be directly analyzed by holding them with tweezers and exposing them to the ionizing gas, or in transmission mode geometry (useful for transmissive samples, such as meshes) . Powders can be mixed with metal particles, adhered to a permanent magnet and exposed to the DART gas. Foam swabs, solid-phase extraction materials, and PDMS coated stir bars, can also be directly placed within the DART ionization region.
In this presentation we will discuss advances from our group in (a) using commercial DART sources to screen falsified artemisinin combination therapy drugs found in Africa, (b) development of an electrothermal vaporization interface for DART, (c) developing an imaging laser-ablation/DART MS system for imaging 2 D-HPTLC plates, and (d) development of microplasma ion sources for ambient ionization as los gas flow DART-alternatives.

Oral Session

Friday, 21st September

Room D

09:00 – 11:00

Session 44: Ambient Ionization

Chair: Jentaie Shiea (National Sun Yat-Sen University, Taiwan)

S44-1000
10:00 – 10:20

Monitoring intra- and inter-day human breath metabolic signatures supports the existence of individual phenotypes

Pablo M-L Sinues[1], Lukas Meier[1], Christian Berchtold[1], Noriane Sievi[2], Giovanni Camen[2], Malcolm Kohler[2], Renato Zenobi[1]

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Keywords:
Breath analysis ; secondary electrospray ionization ; metabolomics

Novel aspects:
Unraveling the existence of individual breath metabolic signatures.

Abstract:

Introduction

The study of changes in the human metabolome towards diseases is a promising area of metabolomic research [1]. However, the reliability of such studies may be obscured by the day-to-day individual metabolic variability. Groundbreaking urine characterization with NMR has suggested the existence of strong individual phenotypes [2, 3] which, moreover, are stable and statistically "recognizable" even after years [4]. These studies emphasize the importance of defining individual metabolite phenotypes for future personalized medicine [3]. The analysis of breath is an attractive metabolomics technique because it is completely non-invasive. Moreover, if conducted on-line, it is rapid and circumvents artifacts due to sample storage/manipulation. In addition, it covers different metabolites than NMR does. Therefore, it is an ideal approach to confirm the existence of such individual metabolic signatures.

Method

4 subjects were asked to breathe at a flow rate of 3.8 L/min through a tube connected to the curtain gas line of a commercial MS. The breath samples encountered an electrospray of water, whereby some compounds in breath were ionized and readily detected. A total of 341 mass spectra collected during four days were analyzed statistically.

Preliminary data

We observe strong intra-day variations of metabolic signatures presumably due to individual circadian cycles. However, in full agreement with prior NMR work [2-4], we also observe a clear inter-individual separation due to the existence of a strong individual "core" signature.

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Oral Session

Friday, 21st September

Room D

09:00 – 11:00

Session 44: Ambient Ionization

Chair: Jentaie Shiea (National Sun Yat-Sen University, Taiwan)

S44-1020 Alternative Desorption/Ionization Processes of Low-Temperature Plasma Probe Ambient Mass Spectrometry

10:20 – 10:40

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Keywords:
LTP, Ionization Mechanisms, Ambient MS

Novel aspects:
This is the first evidence of non-thermal desorption with a plasma-based ambient ionization source.

Abstract:
Ambient ionization mass spectrometry has been an emerging field since its onset in 2004. Of the many different ambient ionization techniques, which desorb and ionize samples from their native environment with little to no sample preparation, nearly half use an atmospheric-pressure discharge for desorption and/or ionization. While little work has focused on elucidation of desorption mechanisms for the various plasma-based ambient ionization sources, many studies have suggested that plasma-based sources rely on thermal desorption as the primary mechanism with no apparent evidence for non-thermal-desorption mechanisms. Furthermore, it is widely believed that thermal desorption is followed by commonly known atmospheric-pressure chemical ionization (APCI) reactions, such as proton and charge transfer from capable reagent ions (formed from ambient air molecules in the discharge) .

The present work demonstrates that non-thermal desorption processes take place with an ambient plasma ionization source in addition to the widely accepted thermal desorption. In this study, a room-temperature plasma, the low-temperature plasma (LTP) probe, was used in conjunction with a Thermo LTQ ion trap mass spectrometer to examine various non-volatile samples. Samples ranged from thermally stable self-assembled monolayers (SAMs) to extremely low volatility organic salts to long-chain polymers. One observed desorption pathway involved breaking of chemical bonds through a high energy transfer from reagent species. For example, a heavily fluorinated decanethiol which forms a monolayer commonly called F-SAM, revealed numerous peaks corresponding to breaking of carbon-carbon bonds in various positions as well as varying degrees of oxidation. The nature of this interaction of high-energy plasma species with surfaces will be further probed by examining the modification of surfaces after exposure to the LTP. Upon LTP-MS analysis of organic salts, the intact molecular cation was almost never observed. Quaternary amines typically revealed loss of the most favorable R-group with subsequent protonation. An additional category of compounds included low-volatility alkyl halides (e.g. thyroxine) , which often revealed only the negatively charged halide in the mass spectrum.

A potential explanation for the responsible desorption process includes electron transfer to the non-volatile samples analyzed, which each have high electron affinities and undergo excessive fragmentation as shown in the mass spectra (common with electron transfer dissociation) . While volatile perfluorinated compounds have been readily shown to ionize via electron transfer under ambient conditions, a significant difference with the present research stems from electron transfer to a solid phase sample, providing the energy necessary to break chemical bonds and release a fragment into the gas phase. Many reagent ions produced by LTP are capable of electron transfer to samples of higher electron affinity, including O_2^- which is abundant in the LTP sampling region. O_2^- has been listed as the likely candidate in other atmospheric-pressure ETD processes and evidence for large amounts of oxidation to the fragments obtained in this study further substantiate this hypothesis. The role of O_2^- as an ETD reagent will be investigated by controlling the ambient environment and limiting the amount of oxygen that can reach the discharge/sampling region. The effect of sample (or substrate) temperature on analyte-ion signal will also be determined to better understand the role of plasma-gas temperature in the desorption of these species.

Oral Session

Friday, 21st September

Room D

09:00 – 11:00

Session 44: Ambient Ionization

Chair: Jentaie Shiea (National Sun Yat-Sen University, Taiwan)

S44-1040

10:40 – 11:00

Analysis of Polar Components in Heavy Oils by Gas Chromatography Electrospray Ionization Mass Spectrometry (GC-ESI/MS) and Multivariate Analysis

Chu-Nian Cheng[1], Yi-Tzu Cho[2], Hung Su[1], Jentaie Shiea[1]

[1]National Sun Yat-Sen Univrsity, Kaohsiung, Taiwan, [2]Yuh-Ing Junior College of Health Care & Management, Kaohsiung, Taiwan

Keywords:
Gas chromatography ; Electrospray ionization ; Heavy oil

Novel aspects:
Gas chromatography electrospray ionization mass spectrometry (GC-ESI/MS) and multivariate analysis were used to analyze polar components in heavy oils.

Abstract:
In the coupled atmospheric electrospray ionization source with gas chromatography system for mass spectrometry (GC-ESI/MS) , after injecting samples into GC injector, volatile compounds underwent GC separation and then individually flew out from the outlet of GC column through carrier gas. These separated analytes mixed with the plume that was generated from an electrospray emitter spraying acidic methanol solution continuously. In this process, ion-molecule reaction carried out that assisted polar analytes to be ionized and formed protonated ions, which was later detected by quadrupole time-of-flight mass spectrometer.
In this study, GC-ESI/MS was demonstrated for the detection of polar components, following with using a multivariate analysis, principle component analysis (PCA) , to differentiate the polar chemicals in heavy oils. The heavy oils used in this study were the engine oils collected from vehicles at different mileage. In the analysis of heavy oils using conventional GC/MS, it was found that the ions of hydrocarbon and other relative compounds suppressed the ions of polar component even after the pretreatment of GC separation. In contrast, our developed GC-ESI/MS reduced interferences of hydrocarbon and other relative compounds due to the feature of the ionization source ESI, which is distinct from electron impact that is commonly used in the conventional GC/MS.
For assessing the applications of GC-ESI/MS in analyzing heavy oils, we collected engine oil samples from vehicles at different driving distances of 0, 100, 300, and 500 kilometers. In sample pretreatment, the samples were mixed and extracted with methanol. The extractants were then diluted with methanol for subsequent GC-ESI/MS analysis, and a series of mass spectrometric results were acquired. In the ion chromatographic diagrams and mass spectral profiles, because the ion signals of hydrocarbon compounds were eliminated, the specific ion information of polar compounds in engine oil samples was obtained. The GC-ESI/MS analyses on each engine oil sample were performed in triplicate, and the results were further processed using PCA. In the grouping of samples, oils collected at 0, 100, 300, and 500 kilometers clearly formed different groups from each other, reflecting their different degrees of polar component generations at varied driving distances.

Oral Session

Friday, 21st September

Room E

09:00 – 11:00

Session 45: Cell Biology / Cellular Pathways

Chair: Renato Zenobi (ETH Zurich, Switzerland)

S45-0900 [Keynote Lecture] Future of Cell Analyses and Mass Spectrometry

09:00 – 09:40

Tsutomu Masujima

Hiroshima University, Hiroshima, Japan/RIKEN QBiC, Suita, Japan

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Keywords:
future analyses of cells, cell biology, mass spectroscopy

Novel aspects:
The future analyses of cells are foreseen by standing on recent development in mass spectroscopic analyses of cells in the world.

Abstract:
When you search the Web-of-Knowledge with the words "Cell " and "Mass Spectrometry ", you can find more than 400,000 papers published since 1964 in which "Biochemistry Molecular Biology " is 11,300papers, "Biochemical Research Methods " 7,300 papers, "Chemistry Analytical " 6,500 papers, and "Cell Biology " is 2,400 papers. The number of papers for methodology oriented and one aiming for biology may be in similar percentage. In any decades, methodological development and its application are usually coupled in idea. Total number of papers shows that biology or cell biology has been one of the big driving force for development of mass spectrometry in the past half century. Here, by reviewing the past researches in biology or cell biology using mass spectrometer, I would like to foresee the future analyses of cells or cell molecular dynamics with my little knowledge.
I would like to foresee the future analyses from another view point, i.e. desires of cell biologist. I think they would like to perform :
1) Direct and exhaustive molecular analysis of cells with easy identification, quantitation and its localization.
2) Cross correlations of gene expressions, transcription, translation and small molecules, including molecular chain correlating or depending reactions and its mapping.
3) Morphological evidence and direct relating of it to above mentioned aspects.
4) Molecular mechanism in macro, micro or even single molecular scale.
Against to these desires, mass spectrometry has been powerful but the power is decreasing in this order. In addition, I made self question "Is it real way of strategy or desire for future cell biology? " Because this kind way of thinking is all standing on substance. Actually, the body, fluid (e.g. cytosol) , and partitions (e.g. Nucleus) etc. of cells are all constructed by molecular substances and are designed very purposefully. However, I still think something is missing for understanding the "life ". It may be one which is not deterministic or dynamic or unstable.
Mass spectrometry is very powerful for substance quantitation and identification, because the mass is a deterministic aspect of substance. However, it may be possible to know the molecular interaction or molecular arrangement force through direct ionization process of whole cell sample, for example. I think mass spectrometry has many potentials which are not considered to be of use for future analyses of cells.
In this keynote, I would like to discuss these potential future analyses of cells by standing on recent development mass spectroscopic analyses of cells in the world.

Oral Session

Friday, 21st September
09:00 – 11:00
Room E

Session 45: Cell Biology / Cellular Pathways

Chair: Renato Zenobi (ETH Zurich, Switzerland)

S45-0940 Telling the Biological Story - Post Processing of -Omics Data

09:40 – 10:00

Michael Kohl[1], Hagen Meckel[1], Maike Ahrens[1], Helmut E Meyer[1], Martin Hofmann-Apitius[2], Martin Eisenacher[1]

[1]Medizinisches Proteom-Center, Ruhr University, Bochum, Germany, [2]Fraunhofer Institute for Algorithms and Scientific Computing SCAI, Sankt Augustin, Germany

Keywords:
bioinformatics, data processing, integrating -OMICS techniques

Novel aspects:
The delineated workflow utilizes the result of the SCAIView text mining software and integrates different -OMICS techniques.

Abstract:
The development of new diagnostic markers and therapeutic targets that aim at both early diagnosis and novel treatment opportunities, are very important tasks of current medical research.
Hence, the PROFILE consortium (www.profile-project.de) addresses these challenges with respect to the most important acute and chronic liver diseases. Liver diseases have extraordinary socio-economic significance, because they are characterized by both high mortality and morbidity. Additionally, due to the central role of the liver, regarding for example metabolism and detoxification, liver dysfunction often has highly significant effects on the function of other organs (e.g. brain, kidneys or the immune system) .
The consortium uses a broad range of state of the art OMICS techniques including Genomics, Proteomics and Transcriptomics, aiming at a more rapid and efficient development of both therapeutic and diagnostic approaches.
However, the huge amount of acquired multidisciplinary data needs to be integrated in a structured manner. Data processing within PROFILE requires the application of adequate algorithms in order to compare and to relate the findings obtained from the participating working groups. Additionally, there is need to develop approaches to extract biologically relevant meaning starting from the pre-processed data.
Here, a software named PROFILE commander (ProfCom) is presented, which facilitates the data analysis of several different OMICS experiments. The software is modularly organized in order to support easy integration of further analysis opportunities. Currently, the ProfCom comprises five modules that can be used independently. However, when connecting in series, the usage of the modules represents a centralized work flow for the data obtained within the PROFILE consortium.
The first ProfCom module deals with evaluating the proteins found by mass spectrometry regarding the reliability of the underlying set of identified peptides.
The second module facilitates conversion into the data formats used by several tools that are applied within the PROFILE consortium. Furthermore, missing information is retrieved from internet resources and then added to the experimental data.
The next module involves some statistical analysis, i.e. the considered biomolecules (i.e. genes or proteins) are ranked in order to detect promising candidates for further experimental validation. Furthermore, multivariate methods are used to differentiate sub-populations within a given data set.
Scientific standards call for relating the own experimental results with the knowledge found in the literature. To this end, ProfCom uses the output of a web based text mining tool (SCAIView) in order to reveal associations between the considered disease and the relevant citations, in a structured way.
Finally, the last ProfCom module relates the findings of the studies, i.e. regulated proteins and genes, to knowledge given by pathway information stored in databases that are available online. The software produces a final output, which can be used directly as input for Cytoscape. Cytoscape is one of the most widely used network analysis and visualization software and can be utilized for the production of publication-ready visualizations and for further analysis (e.g. GO term enrichment analysis via the BinGO plugin) .
The ProfCom is further linked to ProLiC, software developed by the Medizinisches Proteom-Center. ProLiC is a web based tool that enables comparison of an arbitrary number of data sets and calculates the overlap as well as groups the members of the data sets according to several identity criteria.
In summary, the delineated work flow covers a directed data processing, starting with the application of quality criteria to the pre-processed data, up to scientific presentation. Here, performance of the software is demonstrated in the context of hepatocellular carcinoma, the most prevalent cancer of the liver.

S45-1000

10:00 – 10:20

Realtime Molecular Analysis of Single Cell State by Fluorescence-assisted Live Single-cell Mass Spectrometry

Naohiro Tsuyama[1], Hajime Mizuno[1], Takanori Harada[1], Sachiko Date[2], Tsutomu Masujima[1,2]

[1]Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima, Japan, [2]Quantitative Biology Center (QBiC), RIKEN, Osaka, Japan

Keywords:
single cell, nanospray, direct ionization, fluorescent probe

Novel aspects:
Fluorescent probe assisted Live Single-cell MS can clarify the exact nature of cellular state of targeted cell such as redox or molecular diffusion.

Abstract:
Improvement of mass spectrometry allows the volume of samples of biological analysis smaller. Widely used conventional molecular analyses use large numbers of cells which allow detection of rare molecules even with low content, however, give averaged result. As a consequence, phenomena with cell variation or asynchrony are hardly analyzed by these methods. To resolve this issue, we developed Live Single-cell MS which enables targeted cell recovery with a nanospray tip and direct mass spectrometric analysis to detect molecular content of the cell. In this paper we will show two different approaches using fluorescent probes-combined Live Single-cell MS to show cell state related molecular change which cannot be achieved using conventional large scale analyses.

Analyses of cell state related molecular composition
Ordinary microscopic observation only gives morphology or morphological change of target cells. To know cell conditional variation related molecular change such as cellular redox state, we used fluorescent probes. Human EB transformed B lymphocyte apoptosis induced by betamethasone was monitored with dichlorofluorescein (DCF) to clarify cellular level of reactive oxygen species. Cells of high or low oxidative stress were selectively recovered under a fluorescent microscope (OLYMPUS) and analyzed by this method. Conventional LC-MS analysis was also performed to show the advantage of single cell analysis. Among decreased peaks in treated cells, glutathione and its metabolites were gradually decreased after treatment : the intensities of these ions were half of non-treated cells at 1 hour. Whentreated cells were observed under a phase contrast microscope, cell shape was various ; some cells died, and others showed round live shape. Then, cells stained with DCF with bright and dark fluorescent strength were recovered separately with a nanospray tips and applied to SIM analysis in a LTQ-Orbitrap (Thermo Scientific) mass spectrometer. Peaks monitored were *m/z* 308.1, $[M+H]^+$ for reduced form of glutathione, and *m/z* 402.2, $[M+H]^+$ for DCF as a control. Comparison of glutathione level between oxidative stress-high cells and -low cells showed that more than one fifth decrease of glutathione level in stress-high cells was observed whereas the LC-MS data was half. These data indicated that conventional LC-MS method gave an averaged result of heterogeneous cells, whereas our fluorescence assisted-single cell MS gave sharp exact results.

Analysis of dymanic molecular diffusion
Live normal cells have pores called "gap junction " which allow substances including membrane impermeable compounds to pass through between two adjacent cells. Existence and activities of gap junction are usually monitored with fluorescent dyes, for example, Lucifer Yellow CH (LY) loaded into cells. However, no method which can clarify non-fluorescent molecules to diffuse through the junction is existed. We applied the Live Single-cell MS to detect non-fluorescent molecules diffusing through gap junction in normal human fibroblasts in parallel fluorescent staining. Human fibroblasts were microinjected with LY and fluorescence transport through gap junction was monitored periodically. Non-fluorescent molecules, such as homovanillic acid (HVA) and vanillylmandelic acid (VMA) , were co-loaded. A targeted cell was sucked with a Cellomix tip and analyzed similarly. Peaks obtained with SIM mode were extracted and evaluated. LY fluorescence were detected initially in an injected cell and gradually diffused to the adjacent cells. Then, a cell adjacent to the dye-injected cell was analyzed. LY peak of *m/z* 221.5 detected as $[M-2H]^{2-}$ were clearly observed. A cell without LY fluorescence separated from the dye loaded cell was sucked simultaneously, and no LY peak were indicated. Molecules without fluorescence could be also monitored. HVA peak (*m/z* 181.0, $[M-H]^-$) , VMA peak (*m/z* 197.0, $[M-H]^-$) were detected accompanied with LY peak. These results indicated that the Live Single-cell MS can be used to study gap junction mediated molecular diffusion using non-fluorescent molecules.

Oral Session

Friday, 21st September

Room E

09:00 – 11:00

Session 45: Cell Biology / Cellular Pathways

Chair: Renato Zenobi (ETH Zurich, Switzerland)

S45-1020

10:20 – 10:40

Multi-omics Approach to study the effect of rapamycin treatment on a human cell line

Sudha Rajagopalan, Siji Joseph, Guha Nilanjan, Syed S Lateef, Yugandhar Reddy

Agilent Technologies India Pvt.Ltd

Keywords:
multi-omics, pathway, mass spectrometry, microarray

Novel aspects:
Integration of data from multi-omics study to understand pathways affected by a drug treatment

Abstract:
Rapamycin is an immunosuppressant drug that specifically inhibits mTOR activity and cellular hyperproliferation in many cells types resulting in G 1 growth arrest. In this study we illustrate a multi-omics approach that uses genomics, transcriptomics, proteomics, and metabolomics to understand the effects of rapamycin on a human embryonic kidney cell line (HEK293) . HEK293 cells were harvested at 16hrs post treatment of rapamycin or vehicle alone. RNA, miRNA, proteins and metabolites extracted in five replicates from million cells each were pooled and analyzed. The complete genomic status of the cell was interrogated using CGH arrays. CGH analysis showed that HEK293 cells possess large amount of aberrations at the genome level. Eighteen miRNAs were found to be affected by rapamycin treatment. The target entity list of these miRNAs showed good overlap with differentially expressed gene list from the gene expression microarray. For the proteomics analysis, digested peptides from five replicate extracts were pooled and analyzed in five replicates on a QTOF coupled to HPLC chip MS system. Statistically significant differential features between the rapamycin treated and control samples were identified by targeted MS/MS analysis followed by data base search. Metabolites were extracted using Methanol : water : chloroform mixture. Organic portion was analyzed on GC-MS and aqueous portion was analyzed on LC-MS.
Pathway analysis of differentially expressed mRNA, miRNA, proteomics and metabolomics data indicates rapamycin affects several biological processes including translation, protein synthesis, DNA repair, purine metabolism, and stress response. Many of these individual biological responses have been corroborated by observations in other cell lines. This study demonstrates the utility of applying an integrative approach to investigate cellular responses to environmental change by co-analyzing data acquired from complementary technologies.

S45-1040
10:40 – 11:00

Screening and identification of sulfur mustard metabolites from in vitro samples using different LC-MS experiments for new biomarker development

Mia Halme[1], Maija Pesonen[2], Marja Hagström[3], Markku Pasanen[2], Paula Vanninen[1]

[1]VERIFIN, Department of Chemistry, University of Helsinki, Finland, [2]School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Finland, [3]Center of Drug Research, Faculty of Pharmacy, University of Helsinki, Finland

Keywords:
Sulfur mustard, metabolism, biomarker, identification, LC-MS

Novel aspects:
This study represents method for studying sulfur mustard metabolism without animal experiments or samples from human casualties. With these in vitro assays it is possible to trace specific metabolic reactions.

Abstract:
A biomarker is a distinctive biological or biologically derived indicator (e.g. a metabolite) of a process, event or condition. To verify exposure and to link the exposure with the putative health effects of harmful agents, good validated biomarkers of exposure are essential. For the validation of a biomarker, both in vitro and in vivo studies are needed. Validated analytical methods are also essential for the identification, characterization and quantification of a novel biomarkers.

Sulfur mustard (bis (2-chloroethyl) sulfide, HD) is a potent chemical warfare agent and a Schedule 1 chemical on the Chemical Weapons Convention (CWC) [1]. Metabolism of sulfur mustard is complex. It acts as a vesicant and biological alkylating agent causing erythema, edema, blistering, chronic obstructive pulmonary diseases, bronchiectasis, pulmonary fibrosis, and lung and pleural cancer. The immediate tissues affected are the eyes, skin and respiratory tract.

Based on the earlier animal studies, acute urine metabolism of HD includes hydrolysis, oxidation, and conjugation with glutathione followed by b-lyase catalyzed reactions [2]. These b-lyasemetabolites are considered as unequivocal biomarkers of exposure to HD since they have been found both in animal and human samples. A quantitative and fast LC-MS/MS (SRM) method for the determination of two known b-lyase metabolites 1,1'-sulfonylbis-[2-(methylsulfinyl) ethane] (SBMSE) and 1-methylsulfinyl-2-[2-(methylthio) ethyl-sulfonyl] ethane (MSMTESE) from real human urine was validated during this project [3].

Our objective is to study the metabolism of HD by exposing hepatic cell fractions (e.g. microsomes, cytosol or whole homogenate) to develop a platform for finding new possible biomarkers and to confirm the b-lyase pathway in vitro. Incubations were performed using cell fractions, 0.1 M potassium phosphate buffer containing 3.3 mM $MgCl_2$ (pH 7.4), 5 mM glutathione and 0.25 mM NADPH at 37° C. The reaction was started by addition of HD in dimethyl sulfoxide. The reactions were terminated after specific incubation times by addition of cold acetonitrile, followed by vortex mixing. Precipitated proteins were removed by centrifugation and the supernatants were analyzed by LC-MS.

As there are no reference chemicals for the new metabolites, the structural characterization has to be made using different MS techniques. LC-MS analyses of in vitro samples were performed using three different MS detectors: ion trap, triple quadrupole and time-off-flight (Q-TOF). These provided whole range of different data acquisition methods e.g. full scan MS/MS, MS^3, SRM, parent ion scan, neutral loss scan and exact mass measurement for screening and identification of HD metabolites.

It was observed that the metabolism of HD occurs partially differently in vitro than in human or animal studies. For example two different glutathione conjugates were detected (both mono and bi-conjugates). Results and conclusions will be presented.

References

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[2] R.M. Black, K. Brewster, R.J. Clarke, J.L. Hambrook, J.M. Harrison and D.J. Howells, *Xenobiotica* **22** (1992) 405-418.
[3] M. Halme, M. Karjalainen, H. Kiljunen, P. Vanninen, J. *Chrom. B* **879** (2011) 908-914.

Plenary Lecture

Friday, 21st September
11:45 – 12:30
Main Hall

Plenary Lecture 6: R Graham Cooks

Chair: Yoshinao Wada (Osaka MCHRI, Japan)

PL6-1145 Through a Glass Darkly: Glimpses into the Future of Mass Spectrometry

11:45 – 12:30

R Graham Cooks
Purdue University, West Lafayette, IN/USA

Keywords:
Mass analysis ; Ionization ; Instrumentation ; Ion reactions ; Surfaces ; Chirality ; Miniaturization ; Diagnostics

Novel aspects:
A review and preview of the scope of mass spectrometry

Abstract:
Mass spectrometry may be defined, broadly, as the science and technology of ions. As such, it is a subject of enormous scope. The achievements of 20th century mass spectrometry now have come into focus. In rapid succession these include the measurement of mass/charge ratios of ions, the discovery of isotopes, measurements of mass defects, fundamental understanding of ionization and fragmentation mechanisms and the relationship to bond energies. All this was achieved in the first half of the century. In the second half-century, organic mass spectrometry began with applications to petroleum distillates and moved quickly to natural products, steroids, and small-molecule drugs. Gas chromatography /mass spectrometry was a key to early studies of biological systems. A preoccupation with improving mass resolution and mass range became an established theme and different types of mass analyzers were introduced for this purpose. This quest drove biological mass spectrometry and propelled the development of ionization methods that were increasingly successful in handling solid samples (desorption methods) and solutions (spray methods) . Complex environmental and biological samples led to the coupling of liquid chromatography to mass spectrometers and the development of the tandem mass spectrometry for complex mixture analysis. The highpoint of these applications in chemical analysis was the emergence - largely from within mass spectrometry - of the new subject of proteomics. The explicit discovery of ion/molecule reactions at mid-century saw the emergence of a major subject which had affinities with solution-phase reactivity. The study of solvation effects on thermochemistry and reaction kinetics was one important result. Unimolecular reaction kinetics, energy partitioning during unimolecular dissociation, and the dynamics of ion/molecule and later ion/surface collision events opened new fundamental areas of research with wide relevance to physical chemistry.
The likely preoccupations and achievements of current and later 21st century mass spectrometry can only be glimpsed.... "through a glass darkly ". Will the established role of mass spectrometry in chemical analysis be complemented by a significant role in synthesis whether by soft landing of ions onto surfaces or reactive scattering? MS already has a prominent role in surface characterization but is surface tailoring by molecular modification a major future topic? Is there a role for mass-selected ion beams in transfection and other medical processes? Certainly it is already clear that MS has a role in diagnostics. Direct quantitative analysis of whole blood by the ambient ionization method of paper spray is a demonstrated capability for therapeutic drugs at biologically relevant levels. Diagnosis using characteristic lipid profiles allows diseased tissue to be distinguished from healthy tissue. Will such experiments be used intrasurgically? The requirements include automated correlations of the MS data with libraries and reduction in the size of the instrument, but the fact that the measurements can already be made on unmodified tissue makes an affirmative answer likely. If one can make ions in air can one detect them in air, mass-analyze them in air? Can the worst performance parameter of the mass spectrometer the ionization efficiency be significantly improved? Can atomic probe measurements be coupled with mass spectrometry to characterize biological ions? Alternatively, instead of examining ions after landing on surfaces, can the physical and chemical properties of trapped ions be characterized? A beginning has been made using fluorescence resonance energy transfer, electron diffraction and precise thermochemical measurements based on a desolvation enthalpy scale. Surely MS could make a larger contribution to chiral analysis through ion/molecule reactions or the kinetic method. Will array format mass spectrometers take their place beside single-channel instruments? Finally, perhaps most importantly, does the mass spectrometer have a role in the everyday life of everyday people as a personal and household measurement device?

Workshop 1

Monday, 17th September

Room B-1

17:15 – 19:15

Mass Spectrometry of Polymers and Industrial Materials

Organizer: Hiroaki Sato (AIST, Japan)

Keywords:
Structural characterization, High-resolution TOF, Ion mobility, Polymer degradation

Scope of Session:
Mass spectrometry is commonly used as a technique for the characterization of polymeric industrial materials. This session will discuss the recent progress in the structural characterization of polymers (especially for degraded materials) by advanced mass spectrometry such as high-resolution techniques and ion separation techniques.

Abstract:
Polymeric materials have been requested to have versatile physical properties and special functions depending on their utilization. Nowadays the chemical structures of functional polymeric materials become even more complex based on sophisticated molecular design. Just like new instrumentation of MS technology make a significant contribution to the development of biological sciences, the characterization of industrial materials should also be promoted by the MS technologies. In this workshop, we want to discuss the recent progress in the structural characterization of polymers and industrial materials by advanced mass spectrometry.
One of the important topics of polymer characterization by MS is the development of high resolution time-of-flight mass spectrometry (HRTOFMS). A mass of different combinations of polymer constituents (comonomers, end-groups, branching point etc.) increases the likelihood of isobaric interference. The peaks of different chemical compositions with the same nominal mass overlap can be separated by high-resolution MS. In this workshop, the applications of matrix-assisted laser desorption/ionization (MALDI)-HRTOFMS and gas chromatography (GC)-HRTOFMS will be presented and discussed.
Industrial materials are usually mixtures of homologues or isomers, and off course the isomers having same mass cannot distinct even by high resolution mass spectrometry. The application of ion mobility MS (IMS) may be powerful tool for the characterization of such complex materials. Because the ion separation by IMS is based on the differences in three-dimensional conformation, IMS provides another view to the structural characterization of complex industrial materials. In this workshop, the IMS topic with a fine analysis of a mobiligram for petroleum products will be presented and the applicability of IMS to polymer characterization will be discussed.

Workshop 2

Monday, 17th September

Room D

17:15 – 19:15

Hydrogen/Deuterium Exchange Mass Spectrometry

Organizers: Yoshitomo Hamuro (ExSAR, USA)
Rachel A. Garlish (UCB, UK)

Keywords:
Hydrogen/deuterium exchange, Higher order structure, Protein-ligand interaction, Protein-protein interaction, Epitope mapping, ETD fragmentation, Automation, Software

Scope of Session:
Hydrogen/deuterium exchange mass spectrometry (HDX-MS) is an increasingly popular protein characterization method. This session covers both the application and new method development of HDX-MS. The application of HDX-MS includes higher order structure analysis, protein-ligand interaction, and protein-protein interaction, particularly epitope mapping. Method development includes opportunities for higher resolution analysis using ETD fragmentation, data generation automation, and data extracting software.

Workshop 3

Tuesday, 18th September

Room B-1

17:15 – 19:15

Careers in Mass Spectrometry

Organizer: Anthony W.T. Bristow (AstraZeneca, UK)
Chair: John Langley (University of Southampton, UK)

Confirmed topics and presenters:
Academic Career – Rainer Cramer (University of Reading, UK)
Industrial Career – Caroline Pritchard (LGC, UK)
Government Career – Simon Hird (FERA, UK)
Instrument Manufacturer Career – Takaya Satoh (Jeol, Japan)
Preparing for interviews, career options for mass spectrometrists and the global job market in MS – Dave Jones (VRS, UK)

Scope of Session:
The session is designed to outline a variety of career paths that begin and stay within mass spectrometry and also expand beyond a starting point in mass spectrometry. Several international speakers will briefly describe their career paths (for example: academia, industry, instrument manufacturer). These short presentations will outline the choices made during career development, any challenges encountered and the opportunities available in the different career pathways. In addition, there will be a presentation that describes the current job market for people with a background in mass spectrometry and more broadly in analytical science. The workshop will be very interactive, and we actively encourage attendees to question the speakers about their careers and the choices made.

Workshop 4

Tuesday, 18th September

Room D

17:15 – 19:15

Mass++ and MassBank: Tools for Data Processing and Database on PC

Organizers: Satoshi Tanaka (Shimadzu, Japan)
Takaaki Nishioka (NAIST, Japan)

Keywords:
Freeware, Proteomics, Metabolomics, Multi-vendor, Chemical identification

Scope of Session:
Mass++ (mass plus plus) is freeware for viewing and manipulating various types of mass spectrometric data. Mass++ 2.1.0, which was released recently, is easy to use as search engines such as Mascot and MassBank. MassBank is a public repository for sharing mass spectra of small molecules among research community. A bottleneck in metabolomics is to submit a large amount of mass spectra from LC-MS as the queries to database searches manually. Mass++ and MassBank projects have collaboratively developed a tool that relieves the users from the repeated manual works. Two projects will give tutorials of their tools.

Topics 1:
"Mass++: A Visualization and Analysis Tool for Mass Spectrometry"
Satoshi Tanaka (Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation, 1, Nishinokyo-Kuwabaracho Nakagyo-ku, Kyoto 604-8511, Japan)
Abstract: Mass++ (mass plus plus) is freeware for viewing and manipulating various types of mass spectrometric data. Its primary objectives are: 1. To provide essential functionality for proteomics and metabolomics analysis. 2. To support a wide range of vendors' data file formats. 3. To be easily extendible using plug-in technology.
This year we have released a new version of Mass++ (Mass++ 2.1.0), which contains new functionality such as AXIMA file reading, Batch Processing, De novo sequencing, a new peak detection algorithm, statistical analysis, and overlapping trace settings. The new version of Mass++ also makes it easy to identify substances with search engines such as Mascot and MassBank. Previously, MassBank could be used directly from Mass++, but Mascot could not; to use Mascot search, users had to extract peak data and search databases using other software, which was very time-consuming. In the latest version of Mass++, users can easily perform Mascot search on raw data. Mass++ can be downloaded from http://www.first-ms3d.jp/english/achievement/software.

Topics 2:
"Constructing MassBank Database on Laboratory PCs"
Takaaki Nishioka (Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan)
Abstract: MassBank (http://www.massbank.jp) is a public repository for sharing mass spectra among research community. Data shared on MassBank are not placed on the MassBank site, but distributed on the contributor's data servers. Currently 24 research groups contribute from nine data servers. Contributors prepare their mass spectral data in "MassBank Record Format", provide PCs as data servers, and manage their data on their own data servers. Users are able to access to all the distributed MassBank data from the access point, "www.massbank.jp". Thus MassBank project has provided various user's tools to search the data and to prepare and manage MassBank records on their data servers. Recently we transferred these technologies to "In-House MassBank" on Windows PCs. Users are able to build their in-house MassBank databases by depositing their own mass spectral data and the copied MassBank data from the MassBank download site. One of the applications of in-house database is to search user's in-house database with a large set of query data that LC-MS output. Mass++ imports and submits the query data to the spectral search against the in-house database by using MassBank batch SOAP-API. This relieves users from tedious, repeated manual works for the identification of the small molecules detected by LC-MS.

Workshop 5

Wednesday, 19th September

Room B-1

17:15 – 19:15

Path to Next-generation IMS: New Concepts, Advanced Instrumentation, and Leveraging the Ion-molecule Chemistry

Organizers: Toshiki Sugai (Toho Univ., Japan)
Alexandre A Shvartsburg (PNNL, USA)

Scope of Session:
Ion mobility separations coupled to mass spectrometry have become a mainstream analytical tool, and both conventional IMS and FAIMS are available in commercial systems now applied in various areas. This workshop will look beyond established products and methods into qualitatively new approaches that would take IMS to the next level. Topics of presentations and discussion will include multi-stage platforms of much greater specificity and peak capacity, use of ion-molecule chemistry to expand the separation space and modify resolution of specific targets, and various integrative measurements incorporating the IMS dimension.

Abstract:
Over the last decade, IMS coupled to MS has grown into a mainstream analytical tool that now rapidly expands into diverse applications in proteomics, metabolomics, characterization of natural products, and industrial and environmental monitoring. In another major shift, differential or field asymmetric waveform IMS (FAIMS) that exploits the difference between ion mobilities at high and low fields has appeared as a broadly useful approach that is competitive and often superior to conventional IMS based on absolute mobilities at moderate fields. These trends have largely been due to introduction of conventional IMS/MS and FAIMS/MS systems by Waters, Thermo, and AB Sciex. On that trajectory, most high-end commercial MS instruments would in a few years include an (optional) IMS stage. This situation signifying successful integration of IMS into the repertoire of MS and thus analytical science, it is timely to think beyond the current paradigm to take IMS to the next level. Such emerging work will be the focus of our workshop.
The workshop will be appropriately opened by David Clemmer (Indiana Univ.) who had launched the transformation of IMS/MS from a niche physical chemistry technique of academic interest to a widely employed analytical method in 1990-s and forcefully pushed the frontier of this field since. His groundbreaking development of multistage IMS platforms and cyclotron IMS has enabled unprecedented resolving power that would be central to future IMS advances.
Since the invention in early 1990-s and until recently, conventional IMS (in both drift-tube IMS or DMA variants) employed static electric fields only. An innovative direction is use of dynamic fields, notably in the traveling-wave IMS implemented in the Synapt series. Here, Dr. Vidal de Miguel (SEADM) will present a novel IMS configuration employing orthogonal oscillatory and fixed fields. The first prototype already provides reasonable resolution in a compact footprint.
Next, Toshiki Sugai (Toho Univ.) will talk on the development of an ion trap/IMS system that enables one to monitor structural evolution of ions for hours. In particular, evaporation of water droplets and slow thermal deformation of polystyrene particles have been observed. Applications to nanomaterials and the new system with much higher resolving power will be discussed.
The quest for more specific ion separation and identification drives use of IMS to pre-separate isomers for various spectroscopic and chemical selectivity studies. Fuminori Misaizu (Tohoku Univ.) will lecture on the spectroscopy and reactions of isomer-selected clusters. His versatile apparatus coupling drift-tube IMS to ToF MS enables performing photodissociation, collision-induced dissociation, ion-molecule reactions, and negative-ion photoelectron spectroscopy for mobility-selected isomers, exemplified by carbon and silicon cluster cations and anions.
A major aspect of next-generation IMS will be buffer gases comprising vapors that interact with ions specifically rather than inert media that merely retards the ion motion. While such “shift reagents” have helped in some conventional IMS applications, they appear of most value for FAIMS where they generally expand both the overall peak capacity and resolution of targeted analytes. Utilization of vapor dopants is a key feature of the SelexION technology, and the latest highlights in this fast-evolving area will be communicated by Brad Schneider (AB Sciex).
The FAIMS resolving power scales roughly as the cube of field strength that was limited by electrical breakdown threshold. This constraint is essentially lifted in the FAIMS microchips by Owlstone, where fields up to 75 kV/cm permit ion filtering in <100 μs. Richard Yost (Univ. of Florida) will show first findings on the use of these chips with solvent vapors. While resolution gains resemble the effect in full-size FAIMS, the extreme fields in microchips cause intense ion heating that enables rich new chemistry leading to additional separation mechanisms.
A raising MS approach to structural elucidation of proteins and their complexes is chemical cross-linking, but enormous number and diversity of products greatly challenge their detection and identification. Separations by IMS have a major potential here, and Tara Pukala (Adelaide) will describe the use of novel negative-ion cleavable cross-linking reagents in this context.

Workshop 6

Wednesday, 19th September

Room D

17:15 – 19:15

Mass Spectrometry for Food Safety

Organizer: Jentaie Shiea (Nat'l Sun Yat-Sen Univ., Taiwan)

Keywords:
Food safety, Mass spectrometry, Foodborne microorganisms, Mycotoxins, Chemical residues, Pesticides, Food packaging and processing

Scope of Session:
Recent food incidents have drawn the public's awareness of how food supply is regulated and inspected to be deemed safe for consumption. The common food harmful substances include foodborne microorganisms, mycotoxins, chemical residues, pesticides, melamine, plasticizers, and chemical contaminants from food packaging and processing. Only trace amount of these harmful substances is sufficient to cause harmful health effects. Thus, mass spectrometry plays a critical role as an excellent analytical tool to identify food contaminants accurately and efficiently. This workshop explores the recent advances and application of mass spectrometry to food safety, and challenges in food sample preparation.