Poster - 1

Nanomaterial-coated substrate assisted transmission-mode laser desorption for ambient mass spectrometry imaging

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We report a new method that can facilitate the laser desorption process without matrix or nanoparticle treatment step at atmospheric pressure (AP). The use of nanomaterial-coated substrate is excellent platform to induce the desorption process using 532 nm-continuous wave (CW) laser. By using this method, high-spatial resolution atmospheric pressure mass spectrometry (AP-MS) imaging of humid tissues can be obtained. We used the previously developed AP-MS system. One of the most widely used lasers, a 532 nm CW laser, was used as the desorption source. A low-dimensional gold nanoparticle (AuNP) layer was deposited on slide glass and a specimen was placed on the AuNP layer substrate and positioned on the scanning stage for MS imaging. In our proposed AP-MS system, since the laser light was focused on the specimen by the objective lens of the inverted type optical microscope, the laser source was placed under the specimen, so that the laser light was irradiated to the AuNP layer first and the light energy was converted to heat from AuNP layer and transferred to the tissue specimen. Therefore, micrometer-resolution ion images for mouse hippocampal tissue slices were obtained in atmospheric pressure and ambient temperature conditions. AuNP layer substrates can be prepared and stored in advance, resulting in a simplified specimen preparation and a great advantage in preparing fresh tissues faster.

Poster - 2

Polyimide film characterization with chemical analysis (DESI/APGC/HRMS)

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Polyimide (PI) is widely used as an engineering plastic due to its physical properties including; high heat resistance, tensile strength, flexural modulus and electrical insulator. PI products can be customized for various applications including being formed into a film, as a flexible organic light emitting diode (OLED) display material, flexible printed circuit or a lithium ion secondary battery binder. The production of a PI film occurs via the imidization of polyamic acid (PAA) by heat treatment. The PAA precursor is soluble in organic solvents, but not the resulting polyimide, hence only technologies that can perform measurements on solid phase samples can be used post-imidization. To maintain profitability in the specialized materials market, it's necessary to develop polymer materials with specialized functions and launch them promptly. For the development of functional, high performance polymeric materials, it is essential to employ a range of diverse but also complementary analytical techniques. There are two techniques that can be used to investigate both the precursors and the resulting PI film while characterizing the following structure-property relationships; 1. Surface analysis of the PI film with DESI-MS (Desorption Electrospray Ionization-Mass Spectrometry), 2. Structural elucidation of the base polymer by Py-GC/MS (Pyrolysis-Gas Chromatography Mass Spectrometry) using Waters MS^E technology.

Poster - 3

Development of a Lab-on-a-Disc System and Its Application

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A disc for the lab-on-a-disc analysis was designed and constructed to automatically perform sample preparation for quantitative analysis of formaldehyde using LC-MS/MS. This disc was designed to perform a series of well-organized sample preparation steps such as metering, mixing, and filtering, automatically on the lab-on-a-disc system. In addition, an instrument that can perform the lab-on-a-disc analysis was also made in-house, which utilized TTL pulses with PWM modulation to elaborately control the fluid movement and precisely measure a number of real-time revolutions. Further, a high-speed image capturing system was also implemented to monitor fluid movements in high velocities.

Poster - 4

Mechanism Studies of TEMPO-FRIPS Mass Spectrometry

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A method of free radical initiated peptide sequencing (FRIPS) is a radical-based tandem mass spectrometry method in which a radical cation precursor leads to backbone dissociation of peptides upon thermal activation. In this study, free-radical initiated peptide sequencing (FRIPS) fragmentation behavior of *o*-TEMPO-Bz conjugated GGR as a simple model was carefully studied using tandem mass spectrometry experiments and a new theoretical computation approach. We acquired the MS3 mass spectrum of *o*-TEMPO-Bz conjugated GGR and assigned generated fragments based on the suggested mechanism from many research. To carefully investigate the fragmentation mechanism of TEMPO-FRIPS, we compared the products from the case of *o*-TEMPO-Bz conjugated GGR with the products of the intact GGR and *o*-TEMPO-Bz conjugated GRG and RGG. Based on the suggested structures of the products designated from the mass spectrometry, the so-called 'ACE-reaction' algorithm was used for automatic predictions and exhaustive search of low-energy reaction pathways. We could confirm that the main peaks in the mass spectrum were generated by radical-driven fragmentation. In particular, for the $[y_n+2H]^+$ type fragments, we calculated both charge-driven and radical-driven fragmentation mechanism for the confirmation of which mechanism is more plausible.

Poster - 5

Improved Free radical initiated backbone dissociation of peptides conjugated with *p*-TEMPO-Benzyl Succinic Acid

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4-(2,2,6,6,-Tetramethylpiperidin-1-oxyl) methyl benzyl succinic acid *N*-hydroxysuccinimide, so called *p*-TEMPO-Bz-Sc-NHS, is a newly designed peptide-conjugating reagent for free radical-initiated peptide sequencing (FRIPS) that exhibits improved conjugation efficiency with targeted peptides as compared with previously designed conjugation reagent, *o*-TEMPO-Bz-radical initiator. *p*-TEMPO-BZ-Sc-NHS achieves the FRIPS with higher energy collision induced dissociation (CID), which eventually enables the one step MS^3 to elucidate the peptide fragmentation. *p*-TEMPO-Bz-Sc-NHS was conjugated with model peptides and conjugated peptides were analyzed by MS/MS and MS^3 to verify the FRIPS in positive ion-mode. One-step CID enabled the C-O cleavage of *p*-TEMPO-Bz-Sc-C(O)-peptides and dissociated the conjugated peptide complex into *p*-TEMPO radical and •Bz-Sc-peptide radical. Successive beta hydrogen ($H_ß$) extraction of peptide backbone and radical migration dissociated the peptide backbone and produced fragment ions consisting mainly of ***a-***, ***c-***, ***x-***, and ***z-***type ions and those produced from neutral loss. The energetic interpretation of *p*-TEMPO-Bz-Sc-C(O)-peptides were presented in the form of survival fraction to investigate the energetic dissociation of p-TEMPO. Also, LC-MS analysis was conducted on the phosphopeptide from actual protein digest to see if this *p*-TEMPO radical initiator could be applied to future proteomic research with FRIPS.

Poster - 6

Development and Application of Liquid Handler for the Automated Pretreatment of TEMPO-FRIPS

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In analytic chemistry, robust and precise pre-treatment is essential for the effective chemical reactions. Many commercial liquid handling robot have been developed to meet these needs, like reducing the laborious and time-consuming work. In this research, we devised a liquid handling robot named by Dobby 1 suited for the TEMPO-FRIPS reaction by adopting the hardware of Opentrons OT-1. In the Dobby 1, the parts for the framework, such as aluminum profile, Derlin V-slot wheel, belt, pulleys were purchased online. Some parts were fabricated by CNC machine, other parts were 3D-printed. The geometry of the frameworks was optimized by trial and error. In the sense of software, the LabVIEW program was utilized to electronically control a microprocessor, Arduino Mega 2560.

Additionally, for the TEMPO-FRIPS reaction, we are planning to add shaker incubator, heating bed, and robotic arm.

Poster - 7

Major ionization process of electrospray ionization coupled with gas chromatography is atmospheric pressure chemical ionization

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Recently, gas chromatography-electrospray ionization/mass spectrometry (GC-ESI/MS), combining both the excellent chromatographic resolving power of GC and the soft ionization of ESI have been developed and several applications such as H/D exchange, analysis of steroids as TMS derivatives and ionization of polycyclic aromatic hydrocarbons reported. However, to our knowledge, no studies have been reported on the GC-ESI ionization process.

The present study aims to elucidate the ionization process of GC-ESI. The characteristic compounds such as *p*-substituted phenols, enalapril, and POPs, which have different ionization efficiency in ESI and APCI, were analyzed using GC-ESI, GC-APCI (APGC) and LC-ESI. The results of *p*-substituted phenols and enalapril revealed that the major ionization process of GC-ESI is atmospheric pressure chemical ionization. Furthermore, the unused spray solvent and the comparison of copper and stainless steel tubing as interfacing transfer-line strongly supported the possibility of APCI. However, the results of cyclophosphamide (CYC) and other compounds suggested that GC-ESI may have another ionization process along with APCI.

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Poster - 8

Unusual H/D exchange in *meso*-substituted porphyrin investigated by mass spectrometry

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Porphyrin is a well-known organic compound composed of four modified pyrrole subunits. Its two labile hydrogens composed of N-H bonds at the center can undergo fast H/D-exchange under the condition where surrounded by deuterium, whereas other peripheral hydrogens composed of C-H bonds are supposed to be inert to the H/D-exchange. In the present work, however, spontaneous but slow H/D exchanges on peripheral hydrogens have been observed in cationic porphyrins which have methyl pyridinium substituents at *meso* positions. With mass spectrometry, these distinctive and unusual H/D exchange can be directly monitored by observing mass peak shifts in the mass spectra, and their exchange rates can be determined by quantifying mass peak shifts as a function of incubation time. In the present work, we investigated three different porphyrins with regioisomeric methyl pyridinium cations as *meso*-substituents (N-methyl-2-pyridyl-, N-methyl-3-pyridyl-, and N-methyl-4-pyridyl-porphyrins; TM2PyP, TM3PyP, and TM4PyP, respectively) dissolved and incubated in various deuterium containing solvents. The result reveals that deuteriums substituted more than eight peripheral hydrogens for all three porphyrins. Furthermore, H/D exchange differs in accordance not only with the solvents but also with the regioisomeric positions of the methyl group in the *meso*-substituents; TM4PyP undergoes the fastest H/D exchange rate. In addition, collision-induced dissociation experiments and NMR analyses were used to identify the positions of incorporated deuteriums. Density functional theory calculations were also performed to reveal the underlying mechanism of these unusual H/D exchange phenomena.

Poster - 9

Chiral-Selective Aggregation of Serine and Glucose in the Gas Phase

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Many studies on the biomolecular clusters have been done using mass spectrometry because biomolecules can usually form specific self-assemblies that may benefit to build functional biomolecular architectures. Among them, clusters containing amino acid serines have been one of the most interesting systems because of the unique capability of serine to form chiral-selective, magic-number cluster such as serine octamer. Therefore, serine-containing clusters have been considered as possible templates to make chiral-selective biomolecular clusters. In the present work, we investigated the chiral-selective aggregation of serines (Ser) and saccharides (glucose and galactose; Glc and Gal, respectively) under the presence of halide anions (chloride and bromide) in the gas phase by using electrospray ionization mass spectrometry in the negative ion mode. As a result, we found that three serines and two glucose molecules form a unique, highly-abundant cluster with a chloride anion ($[Ser_3Glc_2Cl]^-$) only when the serine and the glucose are of the same chirality, which suggests the presence of a highly stable, homochiral structure of $[Ser_3Glc_2Cl]^-$. The similar chiral-selective cluster was also observed with a bromide anion ($[Ser_3Glc_2Br]^-$), but it is less pronounced than the case with a chloride. Instead of glucoses, galactoses can form a similar cluster with serines and a chloride ($[Ser_3Gal_2Cl]^-$), but its chiral-selectivity is much less than the case with glucoses. Based on the experimental results, theoretical calculations are now ongoing to reveal the structure of $[Ser_3Glc_2Cl]^-$. We expect these results may provide useful knowledge to design chiral-selective functional self-assemblies based on biomolecules.

Poster - 10

Construction of a Quadrupole Ion Trap Time-of-Flight Secondary Ion Mass Spectrometer

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Over the past few decades, time-of-flight secondary ion mass spectrometry (ToF-SIMS) with various cluster ion beam apparatuses has been a powerful instrument for a surface analysis and chemical imaging. Especially the coupling ToF-SIMS with gas cluster ion beams (GCIBs) help us to observe secondary molecular ions from a sample surface for image analysis of biological samples including tissues and cells. Even though it has such great advantages, simple ToF mass spectra have often confused us to assign a peak which several potential candidate molecules might be existed due to the poor mass resolving power of ToFMS.

Therefore, we have been developing a quadrupole ion trap time-of-flight secondary ion mass spectrometer (QIT-ToF-SIMS) for a tandem mass spectrometry. Secondary ions are generated from a sample surface with 10 keV toluene ion projectile produced by 1 kHz laser pulse. The sputtered ions are transferred to the QIT though two sets of electrostatic lens. After accumulating ions in the QIT, the stored waveform inverse Fourier transform (SWIFT) pulse are applied to the QIT for selection of a specific molecular ion. And then UV laser is irradiated onto the selected secondary ion in the QIT for the photo-induced dissociation (PID). The PID-resulting ions are analyzed by ToF-MS.

Hereby, we show examples that different molecular ions with similar mass are separated by the QIT-ToF-SIMS in combination with PID. This instrument would help us eliminate candidates with a confusion come from a similar mass. And furthermore, we expect the PID study for a secondary ion open a chance to see a surface in a new perspective.

Poster - 11

Analysis of OLED materials using LDI-ToFMS

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Organic light emitting diode (OLED) has been widely used in the display industry since Tang and VanSlyke reported the first efficient OLED.[1] By coming into wide use of OLED, it is becoming more important to analyze OLEDs to identify problems related with lifetime, efficiency, luminance and others and clarify the cause of those problems. Studies on OLED issues using laser desorption ionization-time of flight mass spectrometer (LDI-ToFMS) have been reported.[2-4]

OLED materials are easily ionized by laser because most of them have a chromophore which absorb UV light. By controlling parameters such as the wavelength and output power of the laser, we found a best condition that analyzed OLED samples with less damage. Therefore, we can obtain information about the parent molecular ion of the OLED sample by minimizing unnecessary fragment ion signals.

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Poster - 12

Validation of sample preparation method for isotope ratio measurements of Pb and Sr in airborne particulate matter

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Require robust quality control of particulate matter (PM) and the demands on relevant measurement standards are increasing. PM has different chemical composition and isotope ratio depending on their origin, but there are no reference materials collected in Korea. PM reference material for isotope ratio measurements has developed in KRISS. In this study, sample preparation method and method validation has been developed. PM was digested with Single reaction chamber type microwave-assisted acid digestion. Complete dissolution of PM was evaluated after digestion. Pb and Sr was separated from PM matrices. To overcome low recovery of Sr and Mg due to strontium fluoride and magnesium fluoride precipitates formed by reaction with hydrofluoric acid, two-step digestion method was applied. In the first step, the concentrated nitric acid and the hydrofluoric acid were used for dissolution of matrix including silicate. In the second step, fluoride precipitates were dissolved again with boric acid. No residual particulates were observed after centrifugation when optical microscopy was used. In addition, there were no abrupt spike in ion signal chromatogram which was obtained by inductively coupled plasma mass spectrometry (ICP-MS), implying complete dissolution of PM for ICP-MS measurement. Pb and Sr has been separated by ion-exchange chromatography with anion exchange resin or Sr resin (Eichrom). Elemental recoveries were evaluated using SRM 1648a. Mg and Sr recoveries were low in the presence of HF. But After second step digestion, recoveries were improved from ~84% to ~94% in Mg, from ~79% to ~142% in Sr. And KRISS collected PM and analyzed. After two-step digestion, the mass fractions of Sr, Cd, Pb, Mg, Mn, and Fe in the particulate matter were determined by external standard method. Pb and Sr recoveries after separation have been optimized and should be at least 90% for accurate and precise measurements of isotope for Pb and Sr.

Poster - 13

Comparisons of instrumental fractionation models in lead isotope ratio measurements: Standard-sample bracketing, combined standard-sample bracketing with internal normalization, and regression model

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Abstract:

As any isotope ratio measurements by mass spectrometry, there are instrumental isotope fractionations. The actual isotope ratios of lead in a sample can be obtained by, so called, mass bias correction of ion intensity ratios of isotopes. In this study, three mass bias correction models: standard-sample bracketing (SSB), combined standard-sample bracketing with internal normalization (C-SSBIN), and regression model (RM), were applied to correct instrumental isotope fractionation in MC-ICP-MS and the isotope ratios were evaluated. NIST SRM 981 Common Lead Isotope Solution and NMIJ 3681-a Lead Isotopic Standard Solution were used for evaluation of correction models. All the delta-values are in good agreement with the certified values for both correction models. For C-SSBIN, the measured isotope ratios as delta-scale for NMIJ was in better agreement with the certified values than delta-scale applying SSB. For RM which, in principle, is expected to give more accurate isotope ratios compared with SSB or C-SSBIN, an optimized procedure has been developed.

Keywords: MC-ICP-MS, Pb, isotope ratios, instrumental fractionation, mass bias correction

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Poster - 14

LC-MS/MS-based Proteomic Analysis of Synechystis Using Two Phase Samples on Growth curve

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Synechocystis is a type of cyanobacteria, including freshwatrer species PCC 6083 and sea water species PCC 7338. For PCC 6803, many prior studies have already been conducted and are being used in many fields. If PCC 7338 can replaces PCC 6803, positive economic effects are expected.

In this study, PCC 7338 and PCC 6803 were compared based on LC-MS/MS-based DIA method. Identification of the DDA data for each sample was conducted using Comet search and TPP. A quantitative analysis of the DIA data for each sample was conducted using Skyline and MSstat. the library was constructed using the DDA data. For each species, two types of samples were used: the stationary phase and the exponential phase on the gradient curve. Each sample was compared by phase and by species.

The search results of the stationary phase sample were as follows. PCC 6803 was identified with 2184 proteins, of which 588 were unique. In PCC 7338 1794 proteins was identified, of which 168 were unique. exponential phase sample were as follows. PCC 6803 was identified with 1979 proteins, of which 675 were unique. In PCC 7338 1416 proteins were identified, of which 112 were unique. In the search results, the samples from the exponential and static phase showed 82.4% and 70% overlap at 6083 and 7338 respectively, while the quantitative results showed significantly different.

Poster - 15

Development of optimized MS calibration methods for complex mixtures analysis with ESI, APPI, APCI, and LDI

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Ultrahigh resolution mass spectrometry (UHR-MS) is an important tool to study complex organic mixture such as crude oil, contaminated soil extracts, and natural organic matter. UHR-MS provides molecular level information of the organic mixtures. In turn, the molecular level information is important to understand the chemistry and fate of the complex mixture. For MS analysis of the complex organic mixture, employing various ionization methods including ESI, APPI, APCI, and LDI is critical. It is because each ionization method is inherently selective and hence results obtained with various ionization methods have to be combined to cover wide variety of molecules in the organic mixture. For an example, (-) ESI is typically used to study O_x containing and/or non-basic nitrogen compounds and (+) APPI is used to detect aromatic compounds in crude oils. To obtaind the molecular level information, development and application of optimized calibration methods to obtain accurate m/z values is critical. In this study, we propose suitable internal calibrants and calibration conditions for the analysis of three crude oils, three contaminated soil extracts, one humic acid and one fulvic acid samples by (+/-) ESI, APPI, APCI and LDI ionization method. We also optimize the concentrations and mixing ratios of samples and calibrants and present the results. As a result, we obtained experimental data with lower ppm error (<0.1ppm) than the conventional ms calibration data. This study can provide an important guideline for researchers intending to analyze complex mixture by using mass spectrometry with various calibration methods.

Poster - 16

Development of MALDI sample preparation method for reproducible MALDI spectra of synthetic polymers

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MALDI (Matrix Assisted Laser Desorption Ionization) mass spectrometry is a widely used technique for qualitative analysis of synthetic polymers. However, quantitative analysis using MALDI is limited due to the lack of reproducibility in mass spectra. It was recently reported that the plume temperature is an important factor in MALDI spectra of peptides, and it is possible to obtain reproducible MALDI mass spectra by maintaining the constant plume temperature using a laser pulse energy control technique. The same concept could be applied to polymer analysis, but it was difficult to test the technique because the laser pulse energy control technique has not yet been commercialized. Herein we present the sample preparation device to obtain reproducible MALDI spectra with 30% error range using a commercial MALDI-TOF mass spectrometer.

Poster - 17

Deuterium-Free, Three-Plexed Peptide Diethylation for Highly Accurate Quantitative Proteomics

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The deuterium, a frequently used stable isotope in isotopic labeling for quantitative proteomics, could deteriorate the accuracy and precision of proteome quantification owing to the retention time shift of deuterated peptides from the hydrogenated counterpart. We introduce a novel three-plexed peptide "diethylation" using only 13C isotopologues of acetaldehyde and demonstrate that the accuracy and precision of our method in proteome quantification are significantly superior to the conventional deuterium-based dimethylation labeling in both a single-shot and multidimensional LC−MS/MS analysis of the HeLa proteome. Furthermore, in time-resolved profiling of Xenopus laevis early embryogenesis, our 3-plexed diethylation outperformed isobaric labeling approaches in terms of the quantification accuracy or the number of protein identifications, generating more than two times more differentially expressed proteins. Our cost-effective and highly accurate 3-plexed diethylation method could contribute to various types of quantitative proteomics applications in which three of multiplexity would be sufficient.

Poster - 18

Identification and characterization of low-molecular-weight proteins in biological samples using MALDI-FTICR-MS

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Fourier-Transform Ion Cyclotron Resonance (FTICR) analyzer is a mass analyzer with the highest mass resolution up-to-date. By coupling FTICR with Matrix-Assisted Laser Desorption/Ionization (MALDI) technique, ultrahigh peak resolution with dominant singly-charged protein peaks in mass spectrometry (MS) spectra could be achieved, which facilitates the identification of intact protein peaks without the need of tandem mass spectrometry techniques. In this research, we introduce a high-throughput workflow for identifying and characterizing low-molecular-weight proteins (up to 15-20 kDa) of various biological samples (serum, urine, etc...) using MALDI-FTICR-MS with database searching tools. This workflow includes proper sample treatment for MALDI-MS, manipulation of FTICR-MS spectra, protein database search, and confirmation steps. Conclusively, high quality identification using this current MALDI-FTICR-MS approach can become a useful methodology for biomarker discovery, MS-Imaging and other clinical or non-clinical applications.

Poster - 19

Toward the construction of the hazardous accident site gas database using a TD-GC/MS method

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In the accident sites, for example, fire and terror, the detection of gases remaining in the site may provide some evidence for the accidents. These gases can be used as markers to find how the accident occurred. For this purpose, we are developing a thermal desorption-gas chromatography mass spectrometry (TD-GC/MS)-based method that can provide fingerprints of the accidents. Collection of the hazardous gas fingerprints can provide us with data and eventually it will be expanded into the hazardous accident site gas database. As a showcase, we present two specific TD-GC/MS results obtained in two fire sites in the Great Seoul metro areas. The examples show that TD-GC/MS data may provide some fingerprint in the fire sites and this approach will be expanded in the future to construct the accident site hazardous gas database. Moreover, to analyze hazardous gas more specifically, different types of sorbent tubes and GC columns are evaluated.

Poster - 20

Serum lipid signatures of post-hepatectomy liver failure caused by extended hepatectomy using nanoflow UHPLC-ESI-MS/MS

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Post-hepatectomy liver failure (PHLF) is the inability of the liver to perform its normal synthetic and metabolic functions after major and extended liver resection to treat tumors. Despite PHLF, hepatic resection has been used as an effective and safe therapy to treat tumors. Therefore, the development of an accurate but alternate diagnostic means to detect early stage of PHLF would be helpful. The liver is an important organ in lipid metabolism and the serum lipid profiles are changed by the physiological condition of the liver.

In this experiment, the sera were collected from six pigs with only ventrotomy (sham), seven pigs with 70% partial hepatectomy (70% PH), and another seven pigs with 90% partial hepatectomy (90% PH) to induce PHLF at the time points of pre-operation (PO), 14 hours (14h), 30 hours (30h), and 48 hours after the operation. The lipids extracted from the sera were investigated using nanoflow ultrahigh-pressure liquid chromatography electrospray ionization tandem mass spectrometry (nUHPLC-ESI-MS/MS). Based on collision-induced dissociation (CID) experiments, 284 lipid species were structurally identified and 184 lipid species were quantitatively analyzed using selective reaction monitoring (SRM) mode. The six lysoPL and the eleven lipids showed significant differences (> 2-fold, $p < 0.01$) between 70% PH and 90% PH at 48 hours and 30 hours respectively after the operation. Among the lipid species, PC plasmalogen showed significant increases at 90% PH but, TG was found to be significantly decreased at 90% PH.

Poster - 21

Optimizations in simultaneous analysis of fatty acid and other lipid classes using nUHPLC-ESI-MS/MS

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Lipids are the signaling molecules to affect metabolism and perturbations in lipid profiles of phospholipid (PL), glycerolipid (GL), sphingolipid (SL), and fatty acid (FA) are closely associated with the development of metabolic diseases such as diabetes mellitus and cardiovascular disease. When analyzing FA from lipidome sample from plasma or tissues by liquid chromatography with electrospray ionization mass spectrometry (LC-ESI-MS), retention of FA often overlaps with lysophospholipids due to their similar nature in polarity. Moreover, FA can be dissociated from other lipids containing acyl chains when they are introduced to MS.

This study was focused to optimize run conditions of analyzing FA with other lipid classes by nanoflow ultrahigh pressure LC-ESI-MS/MS (nUHPLC-ESI-MS/MS). Modifiers of 0.5 mM ammonium formate and 0.05% ammonium hydroxide were optimized for simultaneous analysis of FA and other lipid classes. When ESI voltage and heated ion transfer tube temperature were examined to determine the effect of unintended in-source fragmentation (ISF), the analysis of FA was not disrupted by ISF of other lipid classes and the degree of ISF from lipids was 15% or less in most cases. ESI volatage of 1.5 and 3 kV for negative and positive ion mode repectively and heated ion transfer tube temperature of 350 °C were optimized for both precursor intensity and degree of ISF. Optimized conditions were applied to examine lipid profiles of plasma from mice.

Poster - 22

Lipidomic perturbations in lipoproteins of patients with postmenopausal osteoporosis by asymmetrical flow field-flow fractionation and nUHPLC-ESI-MS/MS

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As aging population increases, osteoporosis is becoming worldwide issue. Pathological mechanism is unclear, but osteocytes affected by oxidized lipids are expected to be a major factor. Lipids play important roles in signal transmission and various cellular processes. It is important to study relationships between lipids consisting lipoproteins in plasma for the detection of osteoporosis at the early stage.

20 postmenopausal female plasma samples were classified into the groups of patients in which women having osteoporosis(n=10), and control(n=10). Flow field-flow fractionation (FlFFF) is an elution-based separation technique that can sort particles or macromolecules by sizes. FlFFF analysis of patient's plasma resulted in that the relative levels of low-density lipoprotein (LDL) of the patient groups were higher than those of the control with the increase in retention time, while those of high-density lipoprotein (HDL) from patients were not significantly altered compared to those of control. Lipids in lipoprotein fractions collected during FlFFF runs were analyzed by nanoflow ultrahigh-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Overall, 341 lipids from patient's plasma were identified and 264 lipids were quantified. With statistical evaluation, 15 lipid species(6 PC, 1 SM, 3 PEp, 3 HexCer, 1 LPA, and 1 TG) were found to show significant changes(> 1.5 fold and $p < 0.05$) in postmenopausal osteoporosis in comparison to healthy controls.

Poster - 23

Global histidine phosphoproteome using TiO_2 affinity chromatography

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Histidine phosphorylation is a reversible post-translational modification that is known to regulate signal transduction in prokaryotes. However, functional studies in eukaryotes have been largely neglected due to the labile nature of N-linked phosphorylated amino acids. In an effort to help elucidate the heretofore hidden vertebrate phosphoproteome, this report presents a global phosphorylation analysis of *Danio rerio* (zebrafish) larvae. Phosphopeptide enrichment was perfored using a TiO_2 affinity technique. A total of 68 unique phosphohistidine sites were detected on 63 proteins among 1,076 unique phosphosites on 708 proteins. This report provides the first phosphohistidine dataset obtained from zebrafish.

Poster - 24

Metabolic signatures of serum adrenal steroids in 17α-hydroxylase deficiency evaluated by selective LC-MS analysis

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The comprehensive metabolic signatures of adrenal steroids are necessary to understand their pathophysiological functions in adrenal diseases, such as Cushing's syndrome (CS) and congenital adrenal hyperplasia (CAH). An advanced quantitative profiling of adrenal steroids in serum has been developed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). All steroids including 13 glucocorticoids, 9 mineralocorticoids and 5 androgens were separated through an 1.9 μm particle C18 column (50 × 2.1 mm) at a flow rate of 250 μL/min and quantitatively measured by a triple quadrupole MS with electrospray ionization in both multiple-reaction and selected-ion monitoring modes coupled to polarity switching. In method validation, the linearity (r^2) was higher than 0.992 within 0.1 and 500 ng/mL dynamic range, while precision (%CV) and accuracy (%bias) were 1.1 ~ 9.8% and 85.9 ~ 112.1%, respectively. This validated assay successfully recognized 17α-hydroxylase deficiency, which is a rare phenotype of CAH, from healthy and Cushing's syndrome. In a patient with 17α-hydroxylase deficiency, androgen levels were significantly decreased, especially DHEA sulfate (~1/1,000 times), while pregnenolone was increased 10-folds against both healthy and Cushing subjects. In addition, increased mineralocorticoids and decreased glucocorticoids were expectidely observed in a patient. The developed LC-MS method can quantitatively profile biologically active adrenal steroids and inactive sulfate conjugates in a single run to be brining into clinical diagnostic tools.

Poster - 25

Altered androgenic pathways between fetal and adult mouse testes evaluated by GC-MS/MS-based steroid profiling

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Despite decades of studies on gonadal steroidogenesis, the comparative metabolic pathways between fetal and adult mouse testes have not been fully understood. To evaluate the quantitative profiles of biologically active steroids in the mouse fetal and adult testes, the gas chromatography-tandem mass spectrometry (GC-MS/MS) has been developed for selective and sensitive detection of 23 androgens, 7 estrogens, 14 progestagens, and 13 corticoids. All steroids were purified by the solid-phase extraction with Oasis HLB and separated through a stainless steel MXT-1 column (30 m × 0.25 mm I.D., 0.25-μm film thickness) as their trimethylsilyl derivatives. The levels of most androgens quantified were increased in adult testes, while androstenediol was only quantified in fetal testes, which may indicate delta-5 androgenic pathway is dominantly occurred in fetal testis to produce testosterone. In addition, metabolic ratios, representing 7α-hydroxylase activity, in androstenedione and testosterone were significantly higher in fetal testes than that of adult ones. The results will help to understand the developmental steroidogenesis in mouse testis.

Poster - 26

Quantitative analysis of RGB dopant materials by supercritical fluid chromatography coupled with mass spectrometry

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The growth of Organic light-emitting diodes (OLEDs) industry has been remarkable in the display market over the past decade because they are often used in everyday electronics such as TVs, mobile phones and tablet PCs. The basic colors needed are red, green and blue. Various colors are made of the combination of red, green and blue dopant materials. Typically, OLED materials were studied using high performance liquid chromatography (HPLC) with photo diode array (PDA) and fluorescence detector. However, much less research has reported on analysis RGB dopant materials using supercritical fluid chromatography (SFC) and mass spectrometry (MS). SFC can be a powerful technique for OLED analysis because of short analysis time, low solvent consumption, and flexibility in the choice of modifier. MS is a powerful analytical technique to characterize and measure the organometallic compound. In this study, RGB dopant materials were analyzed by SFC coupled with MS. RGB dopant materials were dissolved in THF and analyzed with SFC-MS. Various column combinations were tried to find optimal separation condition. As a result, quantification of ppm level could be routinely done with SFC-MS.

Poster - 27

Middle-down glycoproteomic approach of targeted serum haptoglobin for biomarker discovery in gastric cancer

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Glycans in blood are secreted in the form of glycoprotein and they are changed depending on health condition and diseases including various types of cancers. In particular, the cancer biomarker study based on the site-specific glycosylation of targeted glycoprotein is effective due to high sensitivity and high specificity. Generally, site-specific glycosylation analysis was performed two different ways, proteomic approach using trypsin digestion and LC/MS/MS analysis, and glycomic approach using pronase. However, data processing and interpretation of traditional two approaches were difficult, tedious, and time consuming. Therefore, easy and straightforward method for large sets of clinical samples has always been required. In this study, we developed a middle-down glycoproteomic method for targeted serum haptoglobin (Hp) and analyzed clinical samples for gastric cancer biomarker discovery with relatively simple sample preparation and easy data processing and interpretation. Hp was purified by immunoaffinity chromatography and digested by trypsin. Hp peptides directly analyzed by UHPLC Q-TOF MS system without glycopeptide enrichment and purification. To identify the glycopeptides, *in-silico* Hp glycopeptide library was built by the combination of N-glycan profiling and peptide sequence of Hp. The glycoforms of Hp produced by trypsin can be classified into three glycopeptide groups according to the peptide sequence, and 10, 15, and 14 potential biomarkers ($p < 0.0001$) were found from the three groups, respectively. In order to complement and improve the biomarker, multivariate logistic regression model was applied to potential glycopeptide biomarkers of three groups and the area under the curve (AUC) of combined biomarker was 0.974, 0.931, and 0.980. Middle-down glycoproteomic profiling can be a powerful platform for biomarker discovery with rapid, high-throughput clinical applications.

Poster - 28

Serum and salivary profiles of cholesterols in lipidemia

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Saliva is easy to acquire without regard to time and place as the non-invasive clinical sample. Salivary biomolecules are originally glandular, but they could be also diffused from blood. Due to physiological importance of measuring cholesterol, both serum and salivary cholesterol and their metabolic signatures were generated and compared in patients with lipidemia. A gas chromatography-mass spectrometry (GC-MS)-based quantitative profiling method was optimized and applied to measure 3 dietary sterols and 15 endogenous sterols from 92 patients, who are concerned hyperlipidemia. The GC-MS-based results were also compared with lipid profiles, such as total and LDL/HDL cholesterols and triglyceride. Here, the metabolic signatures of salivary cholesterols is introduced and proposed as an alternative tool for assessing lipid profile in clinical practice.

Poster - 29

Simultaneous quantitative analysis of a large number of vocs with a multitude of isotope-labelled internal standards using a headspace gc-ms.

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Volatile organic compounds (VOCs) are organic compounds with a high vapor pressure at room temperature. VOCs are often included in a variety of commercial products, such as daily life chemicals, packaging materials, building materials, and soils with purpose. As the VOCs and the matrix material contained in the commercial products are very diverse, it is required to develop a simultaneous quantitative analytical methodology that enables one to accurately and reproducibly measure the targeted VOCs in the products of interest. Toward this goal, a headspace-GC/MS was used to perform a simultaneous quantitative analysis of 26 VOCs by spiking a number of isotope-labelled internal standards. A total of 26 compounds were classified by their structure and physicochemical properties, and six internal standards were utilized. This approach with the isotope-labelled internal standards is expected to overcome the so-called “Raoult’s law pitfall of the headspace GC analysis”. In this study, the mixture was diluted to five different concentrations to construct a calibration curve for each type of VOCs. In order to quantitatively analyze VOCs contained in the commercial products, the VOC mixture was spiked into five different products. The recovery of targeted materials was made to investigate the correlation between the internal standard and each substance, and the degree of sensitivity relative to the matrix material was evaluated as well to evaluate accurate and reproducible analysis.

Poster - 30

Analysis of Quaternary Ammonium Compounds (QACs) by Liquid Chromatography-Mass Spectrometry

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After the humidifier disinfectant sacrificed many lives, there has been a growing concern regarding the biocidal chemicals contained in the consumer products we use. Since then, to effectively regulate the chemicals in the consumer products, the Ministry of Environment have begun to strengthen the regulations on the usage of biocides in the consumer products. One of the widely-known biocidal chemicals in commercial products are quaternary ammonium compounds (QACs). QACs are a group of chemicals having a structure of NR_4^+, positively charged nitrogen atom with four alkyl chains covalently connected to it. Although QACs are extensively used in consumer product, such as a fabric softner, how to evaluate their exposure coefficient and toxicity has not been systematically established. In this research, we developed and optimized an analytical method using liquid chromatography tandem mass spectrometry (LC-MS/MS) for the identification and quantification of 3 different QACs standards like benzylakyldimethylethylammonium (BAC), alkyltrimethylammonium (ATMAC) and dialkyldimethylammonium (DADMAC). Also isotope substituted QACs were used for relative quantification of targeted materials in the sample. By adopting the method, those compounds were simultaneously analyzed in less than 10 minutes, and linearity, selectivity, accuracy, and precision of the method were evaluated as well. We plan to identify and accurately quantify QACs contained in various commercial products by applying established analytical method.

Poster - 31

Metabolomic analysis of the glucotoxicity state in pancreatic beta cell

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Glucotoxicity means that pancreatic islet beta cells are exposed to prolonged hyperglycemia, resulting in decreased insulin mRNA, insulin gene transcription factor and insulin secretory capacity. These are known to increase the production of reactive oxygen species (ROS) in the glucose metabolism process, and oxidative stresses resulting from this increase in the function of the cells. In this study, we attempted to verify the metabolism changes by inducing the glycosyltoxic state at the cellular level. Cellular models were obtained from rat pancreatic islet beta cells and cell viability and glucose stimulated insulin secretion (GSIS) were measured. For untargeted metabolic profiling, we performed chromatographic separation using an Ultimate 3000 UPLC system (Thermo Fisher Scientific, San Jose, CA, USA). Reversed-phase separation was performed with an ACQUITY UPLC® BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters, Milford, MA, USA). Then, 0.1% formic acid in distilled water (v/v, mobile phase A) and 0.1% formic acid in methanol (v/v, mobile phase B) were used. Detection of metabolites was performed using an LTQ Orbitrap Velos Pro™ system mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with heated electrospray ionization (HESI) in positive and negative modes. Multivariate analysis using partial least square discriminant analysis (PLS-DA) was performed in Pareto scaling to evaluate the overall differences in the each groups. The metabolomics results showed significant metabolic changes in the glycotoxicity status, which may be helpful for the development of biomarkers that can predict glucotoxicity.

Poster - 32

Comprehensive N-glycosylation profiling of canine serum by nano-LC Chip Q-TOF

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N-glycan attached at the asparagine residue of protein is one of the most important post-translational modification that determined the physiological function and the activity of proteins. Changes of the N-glycosylation have been reported to be linked to the various abnormality of biological system such as cancer or immune disease. Research on the human N-glycosylation has been intensively studied but other mammals is not sufficient yet. In this study, we performed N-glycosylation profiling of canine serum by nano-liquid chromatography/quadrupole-time-of-flight mass spectrometry (Nano-LC/Q-TOF MS). N-glycans in Canine serum were separated by porous graphitic carbon (PGC) chip column followed by the enzymatic cleavages. List of N-glycans were arranged on the N-glycan map that display the systemic display of N-glycan profiles on the basis of biosynthetic network. This comprehensive profiling of N-glycosylation in canine serum will be the millstone for the biomarkers discovery of canine diseases.

Poster - 33

Comparative proteome analysis for 3-dimensional spheroids formation compared to 2-dimendional cancer cell culture

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Spheroids have been known to be associated with the emergence of cancer stem cells characteristics such as tumor recurrence and resistance to chemotherapy. But related mechanism and molecular changes for this is still in unclear. Herein, the comparative proteomes were analyzed using cell model that convert cancer cells into highly carcinogenic 3-dimensional spheroids depending on culture conditions. The ovarian cancer cell, SKOV3 were grown under five conditions, which were 2D monolayer-culture, 4 days and 8 days incubation on ultra-low attachment (ULA) plate and 4 day and 8 day incubation on poly(2,4,6,8-tetravinyl-2,4,6,8 tetramethyl cyclotetrasiloxane) (pV4D4) plate, respectively. Five percentage of SDS was used in the lysate buffer to increase the yield of membrane proteins and S-trap digestion method was applied. The 6-plex tandem mass tags (TMT) as an isobaric labeling strategy, following by fractionation technique by the high pH separation (4 fractions) and the LC-MS/MS platform were combined to gain quantitative values of each protein at once with a minimum instrumental error. A total of 5,306 proteins were quantified and they were shown to be located in the cytoplasm (53%), extracellular space (3%), plasma membrane (9%), nucleus (29%) and other (6%). Through the generalized additive models (GAMs) analysis, 1,468 important proteins were selected (culture condition, incubation time or the combined two factors) and were divided into 4 classes which were common spheroid, different spheroid, ULA-specific and pV4D4-specific markers. And the 161 differential membrane proteins were mainly involved in cytoskeleton organization, membrane biogenesis, adrenergic receptor signaling pathway, low-density lipoprotein receptor particle metabolic process, or so on. The way to make spheroid type of cancer cells in two different ways (pV4D4 and ULA plate) induce changes in the expression of proteins and have their own molecular trajectories from 2D to 3D spheroid shapes.

Poster - 34

Minimization of background contamination of endocrine disrupting chemicals in mobile phase and sample preparation

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Endocrine disruptors (EDs) are highly heterogeneous compounds including various class of chemical groups such as phthalates, phenols, volatile organic compounds, polycyclic aromatic hydrocarbons and pesticides, which are an agent that interferes with normal function of natural hormones in the body. Due to their ubiquitousness and long-term exposure in our daily lives, the effect of these chemicals on health have drawn much awareness and it has become more important to develop analytical method for screening at very low detection levels. However, since EDs are present in many components of lab instruments such as common HPLC solvents and lab water, it is challenging to control contamination of EDs in the analytical system. Also, as the sample preparation proceeds, the contamination of EDs in the sample is increased and, this is one of the most difficult problems in accurately quantifying trace levels of EDs.

In the present study, the isolator column system has been developed to eliminate the background contamination of EDs from mobile phase and the effect of isolator column demonstrated. And the most popular sample preparation methods (LLE and PPT) were compared to investigate the contamination levels of EDs during the sample preparation. The results revealed that the present isolator column system and PPT sample preparation method can minimize background contamination of EDs from mobile phase and sample preparation.

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Poster - 35

Multi-class analysis of endocrine disruptors in human urine by LC-ESI/MS/MS with two consecutive liquid-liquid extraction

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Endocrine disruptors (EDs) are agents that interfere with normal function of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and behavior and highly heterogeneous compounds including various chemical classification such as phthalates, volatile organic compounds, phenols, parabens, polycyclic aromatic hydrocarbons, pyrethroid insecticides and tobacco smoke. It is of importance to develop analytical method that can accommodate a wide range of EDs at very low detection levels.

In this study, a sensitive multi-class analytical method based on ultra-high pressure liquid chromatography-electrospray ionization/tandem mass spectrometry (UHPLC-ESI/MS/MS) with polarity switching (PS) and time-dependent selected reaction monitoring (t-SRM) has been developed for simultaneous quantitation of EDs in human urine. The present study aims to reduce urine sample volume and to integrate sample preparation and analytical method of all ED classes. Urine samples (500μL) were extracted via two consecutive liquid-liquid extraction at different pH values after enzymatic hydrolysis for cleavage of the conjugates. Analyses were performed by UHPLC-ESI/MS/MS with optimized PS, t-SRM, cycle time, dwell time, monitoring time and data points. The calibration curves of target EDs in artificial urine showed good linearity ($R^2 \geq 0.99$) and the validation results were successfully obtained. The present multi-class analytical method has the great potential to be an alternative technique for the simultaneous quantitation of EDs.

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Poster - 36

Quantitative proteomic profiling of ionizing radiation effects in a Mouse bone marrow

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Radiation exposure such as radiation therapy inevitably causes side effects in normal tissues and cells. These side effects are typically inflammatory reactions due to abnormal bone marrow responses. In order to understand the molecular mechanism of these side effects, we tried to identify the differences between Lipopolysaccharide(LPS)-induced sepsis model mice and radiation exposed mice. Sepsis model mice except normal mice and radiation exposed mice were also analyzed by considering the conditions for 4 hours and 24 hours after treatment to observe the change with time. For proteomics analysis, mouse bone marrow of each condition was extracted and mass analysis was performed by obtaining protein from the extracted bone marrow. A TMT-labeling quantification method was introduced for comparative analysis of each sample. A total of 4341 proteins were excavated through three repeated analyzes. Among them, 2,675 proteins containing quantitative information in each sample were used for comparative analysis. As a result, it was confirmed that the sample was exposed to radiation rather than the sample of other conditions, and the change of the proteome of the sample after 24 hours appeared. In particular, we observed changes in NRF2-mediated oxidative stress response. These results will help to understand the mechanism of the inflammatory response caused by radiation exposure.

Poster - 37

Analysis of contamination source of contaminated soil by crude oil using GC×GC-MS

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This study was carried out to identify contamination sources of soil contaminated with crude oil. In order to analyze soil contaminated with crude oil, we tried to optimize the existing domestic and foreign analysis methods. The soxhlet was used as a preparation method and the extraction method was set up in accordance with the sample under various conditions. GC×GC-MS was used for accurate qualitative analysis.

For the qualitative analysis, GC×GC-MS was used to classify normal alkane, cycloalkane, and PAH (poly aromatic hydrocarbon), and the characteristics of each crude oil and characteristics of contaminated soil were studied.

Poster - 38

Comprehensive analysis of persistent organic pollutants (POPs) in human serum and comparison of different mass spectrometric ionization approaches

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Persistent organic pollutants (POPs) are lipophilic substances that were ubiquitous in the environment and toxic to human such as Polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs). Human exposure of POPs can cause adverse effects on endocrine, immune system functions and reproductive physiology, therefore extensive profiling POPs in serum is required in health care. In this study, gas chromatography-atmospheric pressure chemical ionization triple quadrupole mass spectrometry (GC-APCI-MS/MS) and gas chromatography-electron ionization triple quadruple mass spectrometry (GC-EI-MS/MS) were compared for quantification of 86 POPs from a single 200 μL aliquot of human blood serum. The APCI source is the soft ionization process at atmospheric pressure, which results in the abundant formation of molecular ions with very little fragmentation, in contrast to extensive fragmentation caused by electron ionization (EI). Of the 86 POPs analyzed, 63 target compounds (73%) were showed the lower method limits of detection (mLODs) that were 2-50 fold lower in APCI-MS/MS than those determined from the GC-EI-MS/MS. Linearity was achieved in the region ($R^2 \geq 0.99$) from each calibration curve both in APCI-MS/MS and GC-EI-MS/MS. These methods were also compared by performing analysis in 25 real human serum samples.

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Poster - 39

Glycoprotein profiling of gastric cancer-associated fibroblast secretome

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Cancer associated fibroblasts (CAF) play a pivotal role in shaping tumor microenvironment to promote tumorigenesis and drug resistance. Altered glycosylation is one of hallmarks of cancer, however glycoprotein signature associated with CAF-derived secretory proteins remains elusive. In order to identify CAF-specific glycoprotein signature, we firstly optimized glycopeptide enrichment condition. We found that pre-clearing of CM using 3KDa MW cutoff filter could greatly enhance glycopeptide enrichment efficacy compared to non-filtered sample (74% vs. 40%). Next, we sought to comprehensively profile glycoproteins in conditioned medium (CM) of CAF or paired normal fibroblast (NF) from gastric cancer tissue employing LC-MS/MS. We identified a total of 169 CAF-specific N and O-glycopeptides (103 glycoproteins) and 121 NF-specific N and O-glycopeptides (63 glycoproteins) Currently, we are investigating biological and clinical relevance of ~~this~~ these CAF specific glycoproteins. Updated works will be presented.

Poster - 40

Easy charcterisation of biologics using SmartEnzymes from Genvois

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Genovis offers a unique range of rapid and easy to use enzymatic tools primarily for the biopharmaceutical industry and academia. Available worldwide, these SmartEnzymes™ are supplied in innovative formats for the development, production and quality control of biological drugs such as monoclonal antibodies, ADCs, biosimilars and bispecifics. SmartEnzymes are widely used for digestion, de-glycosylation and conjugation and are an ideal companion to popular analytical techniques like LC-MS. In this poster we highlight the most recent additions to the SmartEnzymes portfolio: automated antibody subunit generation using FabRICATOR-HPLC, two tools for O-glycan analysis (OglyZOR, OpeRATOR) and SialEXO 23 for specific release and analysis of a2-3 linked sialic acids.

Poster - 41

Reconstruction of cancer cell homeostatic network through analysis of global proteome and phosphoproteome in CRISPR-Cas9 knock-out cell.

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In cell signaling, most of the kinases promote cell proliferation, survival and migration and are therefore associated with cancer development when overexpressed or activated. As an important key to cancer treatment, signaling pathways regulated by kinases are the targets of most cancer drugs. However, targeted anticancer agents are facing limitations due to their resistance and low popularity and efficiencies.

Four kinase genes (ERK2, PLK1, PIK3CA and PAK4) were edited using CRISPR-Cas9 technology and global proteome and phosphoproteome analysis were performed. Proteins were extracted from these knockout cell lines and tryptic digestion followed by labeling using TMT reagent. We performed global profiling and phosphopeptides enrichment using the IMAC method for the labeled proteins. As a result, a total of 7,500 proteins, 15,000 phosphopeptides were identified by LC-MS / MS. We performed KSEA (Kinase-Substrated Enrichment Analysis) in DPPs and predicted an increase or decrease of kinase by knockout genes. We also profiled GOBP (Gene Ontology Biological Process) and Kegg pathways in which DPPs and DEPs are robust. We performed KSEA using DPPs data and predicted kinases expressed up or down by knockout genes. We also profiled Gene Ontology Biological Process (GOBP) and Kegg pathways enriched with DPPs and DEPs.

This study investigated signal transduction changes at the whole cell level in addition to the already known kinase action point in the knockout cell line and reconstituted the changes in the homeostatic network of cancer cells due to loss of target kinase.

Poster - 42

Analysis of polycyclic aromatic hydrocarbons in particulate matters by using GCxGC/High resolution mass spectrometer

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In Korea and China, particulate matter ($PM_{2.5}$) is very serious environment problems. Among many organic chemicals, polycyclic aromatic hydrocarbons (PAHs) are known to be harmful to humans. PAHs has raised concerns because certain PAHs are classified as probable human carcinogens. Thus, the complex PAH compounds extracted from PM2.5 collected during one month were analyzed to compare their identifications, relative quantities and emission sources. Samples of $PM_{2.5}$ were collected using a high volume air sampler. The 20 cm^2 filter was extracted with 20 mL dichloromethane through sonication twice. Samples were filtered and concentrated under N^2 gas. Comprehensive two-dimensional gas chromatography/high resolution time-of-flight mass spectrometry (GCxGC/HRMS) was utilized to analyze the organic extracts. Approximately, 200 PAHs were separated on the polar and sequencial nonpolar GC columns and identified based on the mass spectral data from NIST and Wiley libraries, and exact mass accuracy (<5 ppm) of molecular ion from high resolution data. The identified organic compounds were different qualitatively and quantitatively between Korea and China. In particular, several PAHs with reference materials were quantitatively analyzed. Thus, it is anticipated that the issues between two countries related about the emission sources will be discussed, based on these objective results obtained for PM2.5 collected in Korea and China.

Poster - 43

Comparisons of RP-RP HPLC separation with different pH and additives for TMT based proteomics

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Most of proteomic samples consist of complex biological matrices so that multidimensional liquid chromatography system has been applied to reduce sample complexity prior to tandem mass spectrometry analysis. The effectiveness of the RP-RP LC separation depends on the different selectivity between the two separation stages, which can be obtained by simply altering the pH and additives of the mobile phase. For the successful characterization of the large-scale proteome, we tried to optimize the conditions of the peptide fractionation using various pH value and buffer type of the mobile phase in the 2D-RP HPLC separation system. Three different types of buffer with different pH and additives were prepared as mobile phase. Peptides labeled with TMT reagent from *E.coli* protein were separated into 42 fractions in the first dimension using each of three buffers. Each fractionated sample was desalted and analyzed by Nanoflow RPLC-MS/MS system. Finally, separation efficiency was evaluated with qualitative and quantitative proteomic results.

Poster - 44

Lipidomic analysis in a fibrosis model of unilateral ureteral obstruction (UUO)

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Except for kidney transplantation - a procedure which is exceedingly dependent on donor-match and availability leading to excessive costs - there are currently no permanent treatments available for chronic renal failure. Moreover, the role of lipid metabolites in the kidney has not been fully understood. Herein we investigate the abnormal lipid metabolism and the mechanism of chronic renal failure in a fibrosis model of unilateral ureteral obstruction (UUO). We analyzed lipids and clarified the lipidomic characteristics in whole kidneys when the mice underwent surgery and were kept in the laboratory animal facility for 7 days and 14 days to induce UUO. Lipid quantification was also performed with multiple reaction monitoring-mass spectrometry (MRM-MS) to study the lipidome changes associated with the fibrosis progression. Through quantitative lipidomic profiling in LC-MRM-MS, 30 and 42 differentially regulated lipids were observed in UUO models induced for 7 days and 14 days, respectively, among a total of 350 quantified lipids. Although levels of cholesteryl ester were significantly increased, levels of phosphatidylinositol(PI), phosphatidylcholine(PC), and phosphatidylserine(PS) were reduced in obstructive kidneys compared to WT kidneys. In this study, we found that lipid metabolism, especially levels of cholesteryl ester, strikingly changed with regard to the progression of fibrosis. Therefore, cholesteryl ester should be further investigated because of its potential target to alleviate UUO and kidney-related diseases.

Poster - 45

Inspection of serum metabolomics profiles in adult asthma

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Asthma in adults is an increasing important health issue. Distinct metabolic pathway and metabolite patterns might be involved in the pathogenesis of adult and elderly asthma. the main goal of this study is to investigate differential alteration of metabolomics profile in the plasma of asthmatics patients and healthy adult. Thirty asthmatics patients (Asthma) and 10 healthy control (HC) between age of 18~45 were enrolled for the study. Untargeted metabolic profile in human serum was monitored by Ultra Performance Liquid Chromatography-Mass Spectrometry technique and applied to PLS-DA model.

Some metabolite including bilirubin, cholesterol fragment, triglyceride and sphingolipid metabolitem, phosphatidylcholine and phosphatidylethanolamine showed significant difference between asthma and control.

Phosphatidylcholine(PC) were increased in group. While TG with polyunsaturated fatty acid were increased in Asthma group, TG with monounsaturated or fully saturated fatty acid were decreased in asthma grouo. Bilirubin which is known as a strong antioxidant was reduced in asthmatic patient group.

This study showed metabolite changes in the plasma of, adult asthmatics patients. Further research is needed to validate their clinical and functional relevance.

Poster - 46

Effect of microbe-drived metabolites on the lipid metabolism in high fat diet-induced obese mouse model

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Obesity is highly concerned as a health problem. For a decade increasing reports were suggested in controlling of host energy metabolism by intestinal microbiota. Metabolites secreted by microbes are considered to be involved in the regulation of obesity by interacting with host metabolism. To find out the microbe derived key molecules in controlling host energy metabolism, we investigated the metabolic differences between effective and ineffective strains.

We selected each two kinds of strain from *Lactobacillus salivarius* and *Bifidobacterium animalis* having effect in loss of high-fat diet(HFD) induced body weight or not. Mice received either normal diet(ND) or HFD with or without these four kinds of microbes. Metabolic profiling were performed with GC-TOF on microbial cell cultured growth media and UPLC-Q-TOF/MS on microbial cells and growth media.

In UPLC-Q-TOF/MS result metabolites from effective and ineffective microbes were clearly divided in PLS-DA score plot. Adenosine monophosphate, Acetyl Co-A, which is correlated with lipid synthesis, UDP-Diphosphate-N-Acetylglucosamine were identified as metabolites differentially expressed between effective and ineffective strains.

Poster - 47

Quantitative analysis of free fatty acids in elaiosome of *Coreanomecon hylomeconoides* Nakai species using UPLC-ESI-MS/MS

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Elaiosome is a lipid-rich fleshy appendage to the seeds of many plant species and known to play a critical role in a mutualistic relationship between plants and ants. In an attempt to elucidate the ecological consequences of beneficial symbiotic interactions between plants and ants, quantitative analysis of free fatty acids (FFAs) was conducted with elaiosomes of *Coreanomecon hylomeconides* Nakai that is endemic to Korea. Elaiosomes were sampled from fifteen individuals per each of seven populations, and FFAs were extracted and derivatized with 3-nitrophenylhydrazine for quantification of seven different types of FFAs in multiple reaction monitoring mode on a UPLC-ESI triple quadrupole instrument. Several statistical analyses including principal component analysis, analysis of variance, and Tukey honestly significant difference test on the concentrations of FFAs were performed to assess significant differences among the seven populations. Interestingly, the hierarchical clustering of the concentration of FFAs from elaiosomes revealed four distinct clusters in the seven regions, which is strikingly different from the known genetic properties of *C. hylomeconides* species.

Poster - 48

Study on Photodegradable Compounds of Expanded PolyStyrene (EPS)

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Recently there is a lot of concern over plastic waste. Since plastic is durable and slow to degrade, the debris of plastic material can be found virtually everywhere. In particular, the problem appears prominently in the ocean. Massive plastic garbage patch found in Pacific ocean vividly show the serious nature of the problem. Expanded polystyrene (EPS), often called Styrofoam, is one of the commonly used plastic materials. The Ministry of Oceans and Fisheries has measured the amount of garbage on the coast of Korea. As a result, plastic waste (including styrofoam) accounts for more than 80% of the garbage found. The most common plastic waste is styrofoam. Styrofoam is the most commonly used buoy in aquafarms such as Seaweed and oysters. Unlike other plastic products, styrofoam easily breaks down, so one buoy can turn into a countless amount of micro-plastic. 19 million styrofoam buoys are used in Korea and 1.6 million styrofoam are replaced annually. It is reported that EPS are susceptible to photodegradation because of its soft nature. However, the fate of the EPS materials especially the compounds generated by photodegradation has not been well characterized. In this study, high-resolution mass spectrometry and liquid chromatography were applied to understand the compounds generated by photo-degradation of EPS. The information obtained in this study can provide a fundamental basis to understand the environmental and toxicological influence of EPS.

Poster - 49

Mass-spectrometric evaluation for in-vitro hepatic metabolism of endocrine-disrupting chemicals

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Endocrine disrupting chemicals (EDCs) are substances that cause changes in the hormone secretion function of the body among harmful chemical substances that are toxic. They are tough substances that can change the health of the offspring as well as their offspring. Endocrine disrupters are also more dangerous because they can affect the organism at much lower concentrations than conventional toxic chemicals and are concentrated through the food chain. They are mostly lipophilic and accumulate mainly in the body fat. Environmental hormones bind to hormone receptors in cells to act like hormones (agonists) or prevent normal hormones from binding to receptors (antagonists).

In current study, we optimized mass spectrometry-based in-vitro evaluation system for T4 (Thyroid hormone receptor agonist). It included S9 fraction development and prediction of metabolized compounds via enzymatic reactions (oxidation, reduction, hydrolysis, and conjugation) corresponding to phase1. The future plan builds a spectrum library of the metabolites and identifies the metabolites in phase2.

Poster - 50

Analysis of Diethanolamine (DEA) and Triethanolamine (TEA) in Han River Based Water Source using LC-MS/MS

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Diethanolamine (DEA) and Triethanolamine (TEA) are used as the main ingredients for surfactant and neutralizer and pH adjuster.Among household goods are widely used in industrial sites due to the nature of these materials.Especially, Diethanolamine (DEA) has been reported to it can affectpregnant women's fetus brain development from experiments of animal test.Nitrosamineis well known to produce a carcinogen is response to a substance of formaldehyde during the product manufacturing process.Triethanolamine (TEA) and also a substance that causes skin irritation and respiratory problems, and controversy over its harmful effects continues.

Therefore, Korea is now standards for supervision of water system emissions through monitoring of various hazardous chemicals, including Diethanolamine (DEA) and Triethanolamine (TEA), which are used in a wide range of industrial sites.

In this study, water samples are collected at regular intervals at certain points within the Han River based on water source, and by conducting qualitative and quantitative analyses of Diethanolamine (DEA) and Triethanolamine (TEA) using LC-MS/MS. The project was conducted to check the possibility of human inflow through oral and skin contact as it was discharged into the Han River basin water source.

Poster - 51

Proteomic analysis of exosomal proteins from rat Schwann cell

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Exosomes are nanometer-sized vesicle secreted by various cell types, especially in membrane proteins rich in biological fluids. Ex-vivo analysis of Exosome is becoming increasingly promising as a non-invasive tool for diagnosing and monitoring diseases and providing a new biomarker discovery platform. It is known that exosomes are present in the nervous system but it is not well known compared to other human organ or tissue studies. There is considerable support for the peripheral nervous system of the peripheral nervous system being directly influenced by Schwann cells (SCs). In order to recover the function after peripheral nerve damage, the neurons have not been identified and have no clue how the SCs contribute to the neural regeneration. Recent studies have shown that in ex-vivo, SCs-derived exosomes significantly influence axon regeneration, but there is limited evidence of the mechanism by which exosomes derived from SCs contribute to axonal regeneration. Furthermore, no studies have been performed on the comprehensive exosome analysis using proteomics techniques in the peripheral nervous system SCs. In this study, we present the first proteomic analysis of SCs exosomes. We have attempted to isolate exosome from SCs using the ultracentrifuge method. The common and specific exosomal markers CD63, CD9, Hsp70 and Hsp90 were identified from the exosome fractions in western blot. Protein profiling analysis was performed on exosome derived from primary SCs using Orbitrap Fusion mass spectrometer. The analysis identified a subset of proteins common to all exosomes such as transport (ESCRT) proteins, tetraspanins, signaling, trafficking, and endosome classifying complexes required for the cytoskeleton. The distinguishing feature found in this assay is that the neurotrophin receptor family p75NTR, TrKc, which is known as the surface antigen of dedifferentiated SCs, is present in the exosome. Also, Ncam1, Gap43 and S100, known as dedifferentiation SCs specific markers, are present in the exosome. Expression of semaphorin 3, plexin A, NRP, IgCAM, ephrin B, and ephrin B receptors, which are known as promoting axonal regeneration factors, was confirmed. The results suggest that exosome derived from SCs plays an important role in supporting axon maintenance and regeneration after nerve injury. The specific exosome protein of the primary SCs identified in this study may provide insight into potential diagnostic biomarkers involved in the disease process and regeneration of peripheral neuropathy.

Poster - 52

Tracing of groundwater nitrogen source driving *Ulva lactuca* bloom using $^{15}N\text{-}NO_3$, $^{18}O\text{-}NO_3$ stable isotope ratios

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Recently, occurance of opportunistic macroalgae bloom (*U. lactuca*) is a globally considered a sign of eutrophication. The aims of this study were to determine the cause of the bloom using multi-isotope techniques($\delta^{13}C$, $\delta^{15}N$, $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$) and investigate why the bloom sustained in the lower reach of the bay. *Ulva* coverd Bangdu bay were measured along 9 line transects(20 sites). Water column samples including seawater, groundwater, aquaculture and agriculture drainage were collected for nutrient and isotope analysis to investigate the flux of nitrogen from the anthropogenic source. *Ulva* tissue was collected and analysed both concentration and isotope values for C, N and metals(Sr). The results showed that anthropogenic nitrogen source, specifically ammonium and nitrate, from the agriculture and aquaculture drainage and input from a nearby groundwater acted as a consistent source of nutrient that enabled the bloom to persist in Bangdu bay, Jeju island. With the modeling approach we found a high range in the estimate proportion of groundwater N, indicating that the mixing models is good tool to reveal contribution of N source. The low concentration of metals and high concentration of N in the tissue of *U. lactuca* mean this algae has the potential to be used as a fertilizer or composted if harvested. Better characterization of tributary $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ by better measurements or a more detailed modeling approach will aid in understanding N-cycle dynamics in estuary ecosystem.

Keywords : *U. lactuca*, $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$, anthropogenic nitrogen source, N-cycle

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Poster - 53

DART-MS/MS for metabolite analysis of small brown planthopper (*Laodelphax striatellus*)

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The small brown planthopper (SBP) (*Laodelphax striatellus*) is one of the most destructive pests of rice. It is widespread in countries such as Korea and China. We conducted metabolic studies to investigate the metabolic mechanisms involved in regional differences between planthoppers. Direct Analysis in Real-Time Mass Spectrometry (DART-MS) promises to be a powerful analytical technique for the high-throughput metabolome analysis of insects. In this study, we used DART MS/MS to find tracers related to the origin of SBP in Chinese and domestic collections. We analyzed 80 SBP samples from various regions (n=5). The use of helium gas at 200℃ was observed to allow deprotonation and detected m/z ~1,500 in the previously in positive ion mode. Our results suggest that 4 candidates were selected and identified using principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA). This research demonstrates that DART MS/MS can be a useful technology for distinguishing SBP from various regions.

Key words: Small brown grasshopper, DART-MS/MS, Metabolomic

Poster - 54

MALDI-Mass spectrometry imaging based investigation of Alzheimer's disease models

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Matrix-assisted laser desorption/ionization Fourier-transform ion cyclotron resonance mass spectrometry imaging (MALDI-FT-ICR-MSI) is a powerful label-free technique for investigating metabolites and determining their biomolecules *in situ*. Alzheimer's Disease (AD) is a progressive neurodegenerative disease that causes memory loss and a decrease in general cognitive functions. Marked by its accumulation of neurofibrillary tangles and beta-amyloid, Alzheimer's is believed to be caused by genetic and environmental factors that changes the amyloid precursor proteins (APP). We performed MALDI-MSI to evaluate lipid changes in two Alzheimer's animal models (5xFAD and 3xTg-AD) within the hippocampus and cerebral cortex of each species, and the data were evaluated for reliability. Compound peaks were observed between *m/z* 100–2,000 using 1,5-diaminonaphthalene (DNA) matrix. Mass imaging data were acquired in positive ionization mode with 50 μm spatial resolution. Our results suggest that MALDI-MSI provides *in situ* label-free analysis of various low-molecular-weight metabolites with high spatial resolution. MALDI MSI will allow potential future applications for the *in situ* identification of biomarkers and disease mechanisms in different research fields.

Key words: Alzheimer's disease models, 5xFAD, 3xTg-AD, MALDI-MSI

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Alterations in lipid profile of an depression model detected by MALDI imaging mass spectrometry

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Long-term administration of tricyclic antidepressants to neonatal mice can lead to behavioral changes and disrupt the stress response system during adulthood. This treatment may therefore produce an animal model for depressive disorders. The clomipramine model (20 mg/kg dose) produces changes in the serotonin or norepinephrine systems through the continuous administration of antidepressants from days 6 to 22. Mouse brains were removed and immediately frozen at −80 °C. Brain slices of 12 μm thickness were produced using a cryostat, the 1,5-diaminonaphthalene (DAN) matrix using HTX-M5 was applied, and the distribution of lipids was compared at a spatial resolution of 50 μm per image pixel using 9.4T Fourier-transform ion cyclotron resonance mass spectrometry imaging(FT-ICR MSI). The ion peaks of lipids (*m/z* 100–2,000) were used to create mass ion visualization. Most of these peaks corresponded to corticosterone. These data show for the first time that MSI is suitable for the visualization of the spatial distribution of an animal depression model. The data may be valuable for research and clinical practice.

Key words: Antidepressants, MALDI-MSI, Clomipramine model

Poster - 56

Differential study of Cell Culture Media to Identify Secretary Metabolites with CE Q-TOF

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We have been interested in secretary molecules from cell when we culture the cell in media because we are able to know the conditions of the cell and what stress is applied to the cell. Moreover, cell metabolites are consisted of most of the secretary molecules including amino acids, fatty acids, and lipids. As we know, we have many difficulties to analyze the metabolites from cell in terms of separation, identification, and quantification.

This note describes how to get more accurate and confident results from well-separated chromatography with Capillary Electrophoresis which can give us more rigid peak separation for polar and non-polar molecules. For Identificaion and Quatification, we need to setup more confident searching workflow with HRMS system which can deliver long-term accuate mass for a long the time to get reproducible results from many of samples without any troubleshootings and more easy quatification results from the system with wide dynamic range for high/low abundant molecules at a time.

Differential analysis for this experiment is conducted by Mass Profiler Professional (MPP) to profile different groups and identify some of biomarkers from the secretary metabolites.

We can expect these kinds of solutions would be next choice to catch up more confident results for metabolomics studies especially for high polar/nonpolor molecules.

Poster - 57

Identification and Quantitation of dimethyl fumarate(DMFu) in dye using Agilent 7250 GC-QTOF

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Dimethyl fumarate(DMFu) is mainly used for the preservation of leather products and the removal of fungi from the distribution of textile products. It has also been used as a preservative for various dyes. However, this DMFu can cause skin diseases such as burns, eczema, allergies, atopy, skin cancer and dyspnea on the skin. Therefore, DMFu is controlled at 0.1 mg/kg in textile products including leather products.

In most cases, ISO/TS 16186 testing method is used. In this method, pretreatment is extraction by sonication with acetone. However, for complicated matrix samples, Florisil refining and filters are required. But, dyes have very complex matrix even after refining and filter pretreatment, and most of them are dissolved in acetone and analyzed directly. In this very complex matrix, when the target component was affected, the Agilent 7250 GC-QTOF was used for precise identification and more accurate quantitation.

Two dye samples which were difficult to detect and quantify during the analysis by GC-MS were dissolved in acetone and analyzed by Agilent 7250 GC-QTOF. With the SureMass function, we were able to confirm the spectrum of overlaid peaks quickly and accurately, and the exact mass value obtained from the high resolution mass supported the more accurate identification. Using the targeted MRM of QTOF, more accurate quantification was possible.

The Agilent 7250 GC-QTOF's SureMass and High Resolution, targeted MRM functions can be very well applied to the qualitative and quantitative analysis of target components that generate matrix interference.

Poster - 58

Validated UPLC/MS/MS method for detection of zolpidem in human plasma and its application to pharmacokinetic study.

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Zolpidem is a non-benzodiazepine hypnotic agent which is used for treatment of insomnia. To compare the PK characteristics of zolpidem in Korean subject, we analyzed the plasma concentration of them. In this work, a rapid, sensitive and selective UPLC/MS/MS method for zolpidem in human plasma was developed and validated following Ministry of Food and Drug Safety(MFDS) guideline. Plasma samples were processed with protein precipitation. Doxazosin was used as an internal standard. Chromatographic separation was performed at 35℃ on a Waters ACQUITY UPLC system using a HSS T3 Column (2.1 mm × 100 mm, 1.8μm). The mobile phase consisted of a mixture of acetonitrile and 5mM Ammonium acetate (pH 3.5 adjusted by formic acid; 40:60 v/v). The flow rate was 0.2 ml/min. The UPLC system was coupled to Mass spectrometer equipped with an electrospray interface (ESI) operated in the positive ionization mode. The multiple reaction monitoring(MRM) mode was used for quantification using target fragment ions m/z 308.17 → 235.12 for zolpidem, and m/z 452.17 → 344.09 for IS. This assay method has been fully validated in terms of selectivity, matrix effect, carry-over, lower limit of quantification(LLOQ), linearity, accuracy, precision, recovery and stability. The calibration curves were linear over the concentration range from 0.5 to 500 ng/mL with r2≥ 0.99. The LLOQ was 0.5 ng/ml. Within-run and Between-run accuracy and precision were within 13% and 5%, respectively. The method was successfully applied to pharmacokinetic study of zolpidem after oral administration in human.

Poster - 59

Quantitative Analysis of Metabolic Markers in Urine Samples from Miscarriage/Pre-term Birth Patients Using LC-MS/MS

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Organization for Economic Cooperation and Development (OECD) reported in 2016 that Korea has recorded the lowest birth rate among 36 countries in OECD. Another birth related problem, so called miscarriage, is a pregnancy loss before 20 gestational weeks of pregnant women. According to Ministry of Health and Welfare of Korea, it has been announced that the rate of preterm birth in Korea has increased double in 2017 compared to the rate in 2000. There have been several researches searching for the possible biomarkers like proteins or lipids that exist specifically in pregnant women with preterm births. In this research we investigated several metabolic markers found in urine samples of pregnant women, which could be used to easily diagnose and monitor possible preterm births. Particularly, metabolites, such as formate, acetate, tyrosine, leucine, and lysine, have been reported as metabolic markers of preterm births and abortion in the previous NMR study. In this research, differences in the quantities of metabolites in urine samples between women with normal pregnancy and those diagnosed with miscarriage/preterm infants are compared. Acetate and formate were analyzed using gas chromatography-mass spectrometry (GC-MS), and leucine, lysine, tyrosine and citrate were analyzed using liquid chromatography coupled with mass spectrometry (LC-MS/MS). Deuterium exchanged internal standards for each metabolite were spiked into each urine sample and creatinine were also analyzed to precisely quantitate the metabolites in each urine samples. Furthermore, 16 different variables from patients (height, weight, experience about PTB n etc.) were considered to normalize the difference in individuals. Overall result was evaluated statistically to verify metabolite's reliability as biomarkers.

Poster - 60

Immunoproteomics approach to discover immunogenic SFTS virus antigen for vaccine and diagnostic kit development

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease in Asia area from 2013. The major clinical symptoms of SFTS are fever, vomiting, diarrhea, multiple organ failure, thrombocytopenia, leucopenia and elevated liver enzyme levels, showing its fatality rates ranging from 12% to as high as 30%. SFTS virus is a phlebovirus in the family of Bunyaviridae, and consist of 3 gene segments, large (L), medium (M) and small (S). And 6 proteins have been identified—an RNA dependent RNA polymerase (RdRp), a glycoprotein precursor (M), a glycoprotein N (Gn), a glycoprotein C (Gc), a nuclear protein (NP) and a non-structural protein (NSP). In this study, using immunoproteomics approach coupled with high resolution LC-MS platform, several highly immunogenic SFTSv antigens were discovered. Those antigens are expected to be applied in vaccine development and rapid detection kit development. Also, immunoproteomic approach shows its possibility as a useful tool for antigen discovery.

Poster - 61

Method development of trace amount of nitrogen mustard metabolites for GC-TSQ

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Nitrogen mustards which are vesicant are prohibited from production, stockpile and development by the chemical weapon convention. The Chemical Weapons Convention has been supervised using a chemical weapons verification system based on chemical analysis such as gas chromatography mass spectrometry. Samples for chemical weapons verification can be classified into environmental samples and biomedical samples. A biolomedical sample refers to a sample which is excited from a living body, such as human urine and blood, and the analyte is a metabolite of chemical warfare agents(CWAs). Metabolites of CWAs in biomedical samples are characterized by a trace level of ppb levels. In the case of Nitrogent mustards, hydrolysis occurs rapidly when introduced into the human body and the produced ethanol amines are major metabolites. In this study, we developed the MRM method for the GC-TSQ analysis of trace amount of nitrogen mustard hydrolysis products with derivatization

Poster - 62

Study on method development of trace amount of sulfur mustard metabolites for GC-TSQ

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The blister agents containing sulfur are prohibited from being produced, stored and developed by the Chemical Weapons Convention. Compliance with the Chemical Weapons Convention is monitored by the verification system aimed at obtaining evidence of chemical weapons in the sample through chemical analysis. Samples subject to chemical weapons validation may include biomedical samples such as urine or blood collected from exposed people by chemical agents. Tandem mass spectrometry is mainly used for these biomedical samples because there is evidence of very small amounts of chemical agents. At present, the test for vesicant agents in biomedical samples is mainly performed for HD. However, there are various other substances besides the sulfur-containing vesicle agents, and because of the characteristics of the microanalysis, they cannot be detected unless the analytical methods for each are established. This study deals with analytical methods that can analyze the metabolites of trace amounts of sulfur mustards other than HD using GC-TSQ.

Poster - 63

Development and validation of LC-MS/MS method for the determination of trimethylamine N-oxide (TMAO) in human plasma

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Trimethylamine N-oxide (TMAO), gut microbiota-dependent metabolite, was found associated with cardiovascular disease. To investigate correlation between elevated plasma TMAO levels and increased risk of cardiovascular disease, a sensitive and accurate quantitative method for determining TMAO in plasma is necessary. Thus, liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to quantify TMAO in human plasma.

TMAO were extracted from human plasma by protein precipitation and analyzed by LC-MS/MS with electrospray ionization in the positive ion mode. The limits of detection and the limits of quantification were 25 ng/mL. Intra-assay imprecision of TMAO quantification was evaluated from 5 replicates measured in a single series and inter-assay imprecision was obtained from 4 different assays over 4 days using four different concentrations of quality control materials spiked with TMAO. Intra-assay coefficients of variation (CVs) (n = 5) were 1.2–4.2% and inter-assay CVs (n = 4) were 2.1-6.0%. It was also confirmed that repeated freezing and thawing (three cycles) of plasma samples, spiked with TMAO at two different levels, did not affect their stability; mean percent recovery against spiked concentrations was 98.6 and 93.5%.

This method was sensitive and accurate enough to determine concentrations of TMAO in human plasma and will be very useful to further study the cardiovascular disease related with TMAO and other related factors in vivo.

Poster - 64

Automated derivatization of neurotransmitters in plasma extracts for liquid chromatograph tandem mass spectrometry

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Neurotransmitters are endogenous chemicals that enable neurotransmission. It is a type of chemical messenger which transmits signals across a chemical synapse, such as a neuromuscular junction, from one neuron to another "target" neuron, muscle cell, or gland cell. In vivo neurochemical monitoring is important in neuroscience because it allows correlation of neurotransmission with behavior, disease state, and drug concentrations in the intact brain.

We examined Auto-precolumn derivatization for LC-MS/MS method that utilizes benzoyl chloride for determination of the most common low molecular weight neurotransmitters. Auto-precolumn derivatization, the benzoyl chloride is derivatized before injection, and then the reaction products are separated and detected. This reaction is virtually instantaneous at room temperature and increases sensitivity by up to 1,000 folds.[1)]

In this work, we report the process of blood sample preparation and Auto-precolumn derivatization using benzoyl chloride method followed by LC-MS/MS analysis. This analysis system set-up does not require any additional hardware for sample pretreatment except for pretreatment function built into autosampler.

Reference.

1) P. Song, Robert T. Kennedy et al. Anal Chem. (2012) 84, 412-419

Poster - 65

Hydrogen deuterium scrambling in Nano-ESI TEMPO-FRIPS tandem Mass Spectrometry

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Hydrogen/deuterium exchange coupled with mass spectrometry (HDX-MS) is a well-known method to understand the structural dynamics of the proteins. In general, the deuterium incorporation is estimated at the peptide level by pepsin digestion. Gas-phase fragmentation of the deute

rium exchange peptides in tandem mass spectrometers provides information of deuterium content at a residual amino acid level. The major challenge in this method is the hydrogen scrambling, which takes place during the vibrational activation of the gas-phase ions. Several MS/MS techniques were studied to reduce the extent of scrambling out of which ETD and ECD had proven to have a minimal scrambling than other techniques. Here, in this work, we employed a different fragmentation approach, Tempo assisted free radical-induced peptide sequencing (FRIPS-MS), to investigate the degree of scrambling in a few customized peptides. Our results showed that under controlled conditions using FRIPS-MS, the extent of scrambling is significantly low compared to the collision-induced dissociation.

Poster - 66

Metabolsim and pharmarcokinetic studies of carisbamate in rat using liquid chromatography-quadrupole time-of-flight mass spectrometry

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Carisbamate is an antiepileptic drug which also has a braod neuroprotective activity and anticonvulsant reaction. In this study, a liquid chromatography-quadrupole time-of-flight mass spectrometric (LC-qTOF-MS) method was developed and applied for the determination of carisbamate in rat plasma to support preclinical ADME/PK studies. The method consisted of protein precipitation (PPT) using acetonitrile for sample preparation followed by LC-qTOF-MS in the positive ion mode for analysis. A quadratic regression (weighted 1/concentration^2), with an equation $y=ax^2+bx+c$, was used to fit calibration curves over the concentration range of 9.05~6600 ng/mL for carisbamate. The qualification run met the in-house acceptance criteria of ±25% accuracy and precision values for discovery non-GLP study. This method was successfully applied to the pharmacokinetic and semi-mass balance studies of carisbamate in rat. Carisbamate (1, 5 mg/kg) was administered intravenously and orally to SD rat for pharmacokinetic and semi-mass balance studies. Non-compartmental analysis was used to evaluate the pharmacokinetic parameters of carisbamate. As the results, the bioavailability is close to 100% and clearance value was about 4 mL/min/kg, which was low clearance, considering rat hepatic blood folw.

Poster - 67

Metabolic characterization of Mertansine (DM1): in vitro metabolic stability assessment and metabolite identification in various species by using liquid chromatography-mass spectrometric (LC-MS) method.

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Kadcyla® is the first HER2-targeted antibody-drug conjugate (ADC). It is a single agent with 3 components: antibody, cytotoxic agent (DM1), and linker (MCC). If ADC's linker is unstable, cytotoxic agents can be released in the systemic circulation rather than in the target. The released cytotoxic agents can be metabolized in the liver. Therefore, metabolism studies on cytotoxic agents of ADC will be helpful to predict what happens to the released cytotoxic agents in the systemic circulation.

The purpose of this study is to develop a specific LC-MS method for DM1 to determine metabolic stability and to identify metabolite profiles of DM1 in liver microsomes and S9 fractions. Metabolic stability and metabolite identification experiments were conducted by incubating DM1 in liver microsomal and S9 fraction (at pH 5.0 or 7.4) system. The incubated samples were then analyzed using the LC-MS method developed in house. In metabolic stability study, DM1 showed low stability under the incubation conditions of liver microsomes and S9 fraction (at pH 7.4) at 37°C for 30 minutes. On the other hand, the level of DM1 remained constant over time in S9 fraction (at pH 5.0).

The matrices containing major metabolic pathway of DM1 was liver microsomes and S9 fraction (at pH 7.4) and major metabolite was the loss of $C_7H_{11}O_2NS$ from the parent drug (DM1). Therefore, a novel LC-MS method was successfully developed and applied to evaluate the metabolic stability and identify the metabolite of DM1.

Poster - 68

In vitro catabolic identification of antibody drug conjugates with non-cleavable linkers or cleavable linkers using liquid chromatography-quadrupole time-of-flight mass spectrometry

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Antibody-drug conjugate (ADC) consists of an antibody, linkers and chemical drugs (payloads). The linker stability of ADC is very important in terms of toxicity and efficacy. If the linker is unstable, payload is not likely to target the cancer cells and can be a threat to normal cells. Kadcyla® (Trastuzumab emtansine; T-DM1) is an ADC in which DM1 (chemical drug) is conjugated to trastuzumab (antibody) via MCC (linker), a non-cleavable linker. Adcetris® (Brentuximab vedotin) is an ADC in which MMAE (chemical drug) is conjugated to brentuximab (antibody) via mc-vc PAB (linker), cleavable by cathepsin B.

In this study, we performed experiments to identify catabolites of two ADCs with two different linker types in S9 fractions and microsomes of the various species (mouse, rat, monkey, dog and human) using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF-MS) method. As a result, Kadcyla® was catabolized in the forms of payload, linker-payload and linker-payload with various amino acids in S9 fraction (pH 5.0) and payload and linker-payload in S9 fraction (pH 7.4) and microsome, while Adcetris® was catabolized only in payload form in S9 fraction (pH 5.0 or 7.4) and microsome.

Poster - 69

Metabolic stability of auristain e in various species matrices and its metabolite identification using liquid chromatography–tandem mass spectrometry

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MMAE is an inhibitor of tubulin polymerization, called auristain E. The toxicity of MMAE itself is so high that it has been developed mainly in forms of the conjugated antibody through a linker. Like Adcetris (brentuximab vedotin). Due to the linker stability, MMAE can be cleaved from Adcetris before reaching the target, resulting an off-target toxicity. Therefore, it is important to identify the metabolic stability and the metabolic profiles of MMAE in vivo.

We developed an LC-QTOF-MS method to analyze MMAE, and the method was well applied for the identification of metabolic stability and metabolites of MMAE in various species matrices.

Experiments were conducted by incubating MMAE in liver microsomal and S9 frictions (at pH 5.0 7.4) system from five different species (mouse, rat, dog, cyno and human). MMAE was labile in microsome and moderately metabolized in S9 fraction in a species-specific manner. A total of 25 metabolites were observed from the microsomes but less metabolites were found in S9 fractions the metabolite identification studey. In conclusion, a novel LC-MS method for MMAE was developed and successfully applied to figure out its metabolic stability and metabolite identification.

Poster - 70

Urinary Metabolomic Profiling to Discover Potential Biomarkers of Acute Cellular Rejection in Kidney Transplant Recipients

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Acute cellular rejection (ACR) is one of the most common complications after kidney transplantation. To improve early renal allograft function, it is important to develop a noninvasive diagnostic method for ACR. However, current diagnostic methods for ACR have limitations such as invasiveness, sampling error, or nonspecificity. To complement the shortcomings of current diagnostic methods, thus, it is important to develop a noninvasive, more specific diagnostic method for ACR. This study aims to explore potential noninvasive urinary biomarkers to screen for ACR in kidney transplant recipients using untargeted metabolomic profiling.

Urinary metabolites, collected from kidney transplant recipients with non-rejection (NR) or ACR episodes, were analyzed using liquid chromatography-mass spectrometry (LC-MS). Statistical analysis revealed the differences in urinary metabolites between the two groups. ROC curve analysis showed the best performance of the training set (AUC, 0.926; sensitivity, 90.0%; specificity, 84.6%) using a panel of 5 potential biomarkers: guanidoacetic acid, methylimidazoleacetic acid, dopamine, 4-guanidinobutyric acid, and L-tryptophan. The diagnostic accuracy of this model was 62.5% for an independent test dataset.

Overall, LC-MS-based untargeted metabolomic profiling is a promising method to discriminate between ACR and NR groups. Our model, based on a panel of 5 potential biomarkers, needs to be further validated in larger scale studies.

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Poster - 71

Untargeted metabolomic analysis to characterize exercise-induced metabolite changes associated with myocardial ischemia

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Myocardial ischemia (MI) occurs when the blood flow to heart muscle is decreased by partial obstruction of coronary artery. Exercise electrocardiography (ECG) is used to diagnose ischemic conditions by immediately increasing exercise load. Patients suspected of coronary artery disease may experience symptoms such as chest pain and the occurrence of S-T segment depression during exercise testing. This may induce MI and result in metabolism change in the body.

For untargeted metabolomic profiling, plasma samples were collected from 19 MI patients, who were diagnosed through treadmill test and coronary angiography, before and after treadmill test. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) in both positive and negative ion modes for the analysis of plasma samples. The score plot of orthogonal partial least squares-discriminant analysis (OPLS-DA) showed clear separation between two groups in both positive and negative mode. Mass features satisfying the criteria of variable importance in the projection (VIP) score ≥ 1.0 were considered significantly. To identify these features, we searched various metabolite databases and compared their MS/MS fragmentation patterns to those of authentic standards. We also used other analytical instruments including a triple quadrupole mass spectrometer for precursor ion scan and a quadrupole time-of-flight (QToF/MS) for better MS/MS analysis. The results achieved by this research may provide useful information for the early detection of MI.

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Poster - 72

Liquid chromatography - triple quadrupole - time of flight / mass spectrometry assay for the evaluation of metabolic profile and pharmacokinetic properties of omeprazole in mouse brain and plasma

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A simple and sensitive liquid chromatography – triple quadrupole – time of flight/mass spectrometry (LC-QTOF-MS) assay has been developed for the evaluation of metabolism and pharmacokinetic (PK) properties of omeprazole in mouse. A simple protein precipitation method using acetonitrile was used for the plasma and brain tissue sample preparation and the pre-treated samples were separated by a reverse-phase C18 column. The calibration curve was evaluated in the range of 3.02 ~ 2200 ng/mL and the quadratic regression (weighted 1/concentration) was used for the best fit of the curve with a correlation coefficient ≥0.997. The in vivo PK study was conducted through three types of administration pathways (intravenous [i.v], oral [p.o] and intraperitoneal [i.p]) in mice and the results showed that the p.o and i.p bioavailability were 5.56% and 51.97%, respectively as a mean value, with a low to moderate systemic clearance. The brain omeprazole concentration was also quantified and the result was used for the calculation of brain to plasma drug ratio. In addition, in vivo brain metabolite profiles through i.v, p.o and i.p dosing in mice were also explored. A total of 25 metabolites were observed in our experimental conditions and the metabolite profile was different among three types of administration pathways. As far as we know, there has been no report on the brain to plasma ratio of omeprazole or the metabolite profile of omeprazole in the brain tissues. Therefore, this result would be very useful to better understand the pharmacokinetic and metabolism properties of omeprazole in mice brain tissues.

Keywords: Omeprazole, LC-QTOF-MS, brain to plasma ratio, brain metabolite profile

Poster - 73

A novel and simple LC-MS/MS method for simultaneous determination of lansoprazole, amoxicillin, and clarithromycin in human plasma and its application to a pharmacokinetic study

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A Prevpac (lansoprazole, amoxicillin, and clarithromycin) is a combination of three medicines used to treat infection with Helicobacter pylori (H. pylori) bacteria which can cause peptic ulcer disease. The aim of this study is to establish and validate a simple and accurate liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of lansoprazole, amoxicillin, and clarithromycin in small volume human plasma. Analyses were performed using an Agilent 1200 HPLC apparatus directly connected to an Applied Biosystems 4000 Qtrap® LC/MS/MS system. The samples prior to the injection were precipitated with acetonitrile. The chromatographic separation of analytes under gradient conditions was performed in mobile phase consisting of ACN/MeOH (2/1, v/v) and 0.05% acetic acid buffer on a Zorbax eclipse C18 (100 × 3.0 mm, 3.0 um particle size) column. The method was fully validated by analyzing QC samples prepared with concentrations across calibration range (50 - 3000 ng/mL, 0.1-30 ug/mL, and 0.05-10 ug/mL for lansoprazole, amoxicillin, and clarithromycin, respectively). The described LC-MS/MS method is accurate, simple and reliable with a wide calibration range and also shortens time-consuming sample preparation, resulting in significant improvement in throughput analysis. Therefore, this method seems to be suitable for bioequivalence and pharmacokinetics study of lansoprazole, amoxicillin, and clarithromycin.

Poster - 74

Method optimization of protein extraction from formalin-fixed, paraffin-embedded tissue for global proteome analysis using liquid chromatography coupled with high resolution mass spectrometry

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Formalin-fixed paraffin embedded tissue is an internationally standardized tissue preparation method for morphological pathologic examination using immunohistochemistry in the pathology department because it preserves the tissue and cell morphology of the main specimen. It have been becoming more important as a repository of resources for translational research such as the development of biomarkers, the discovery of new drug candidates, the decision of treatment policy and the preclinical testing of new drugs. In this study, we optimized protein extraction procedure from FFPE of thyroid nodular hyperplasia (NH) tissues as a first step for global proteome analysis using LC-MS platform. First, paraffin was removed using heptane and the proteins were extracted from the formalin-bound proteins by breaking methylene bridges at the presence of high temperature and detergent. Extraction of proteins from FFPE was tried six time for a sample and the protein extraction efficiency for each step was measured. The optimized results from this study can be applied perform proteome analysis of FFPE tissues of various diseases and FFPE proteome analysis can be a useful platform for diagnosis and disease mechanism research as a means of personalized medicine.

Poster - 75

In vitro and in vivo metabolite profiling and identification of sulfasalazine by stable-isotope labeling and liquid chromatography-time-of-flight mass spectrometry

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Metabolite profiling and identification are play a critical role in the drug discovery and development stages to identify the stability of new chemical entities as well as toxicity-related species selection. However, it is sometimes challenging to determine the exact structures of metabolites due to low peak intensity and isotope peak issues of product ions. To overcome these difficulties, an approach using a mixture of stable isotope-labeled compound and non-labeled compound simultaneously was explored in this sthdy. Sulfasalazine (SSZ) and deuterium substituted-sulfasalazine (D4-SSZ) were used as tool compounds in this approach. First, the optimization process was conducted for the optimal peak intensitiy ratio of SSZ : D4-SSZ (1 : 1). And then, the mixture of SSZ and D4-SSZ was incubated with rat liver microsome to identify in vitro metabolites. Plasma and urine samples were also collected from rats orally dosed with the mixture and in vivo metabolites were analyzed as well as to correlate the in vivo metabolites with the in vitro ones. Metabolite profiling was conducted by liquid chromatography-time-of-flight mass spectrometry (LC-TOF-MS/MS) using independent data analysis (IDA) and product ion modes. In vivo metabolites were investigated and verified by comparing metabolites produced by SSZ with metabolites produced by D4-SSZ. These results indicated that oxidation and glucuronidation were main metabolic pathways in vitro metabolism using liver microsome. Also, a total of 5 metabolites were identified in vivo samples and all metabolites were characterized by comparing the product ion patterns of SSZ and D4-SSZ. This study suggests that metabolites identification using the stable isotope-labeled compound and non-labeled compound simultaneously is useful to conduct metabolism studies quickly and effectively with better-confidence.

Poster - 76

Plasma stability of brentuximab vedotin and search of its catabolism and metabolism

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Antibody-drug conjugate (ADC) is composed of an antibody, chemical drugs (payloads) and linkers for the delivery of payloads to the target. Brentuximab vedotin is composed of brentuximab (antibody), valine-citrulline (vc, linker) and MMAE (payload). If the ADC is unstable, the amount of released payloads will be increased in the systemic circulation, resulting in the increased drug toxicity. Assessment for the in vitro stability of the ADC in plasma makes it possible to predict the stability in the systemic circulation. This assessment also allows the prediction of various metabolites/catabolites associated with the ADC. In this study, a novel immunocapture LC-QTOF-MS assay was developed to determine the stability of ADC in plasma and to explore catabolism and metabolism of ADC. Also, antibody-conjugated drug (acDrug), total antibody (tAb), free payload and metabolite/catabolite were measured. As the results of in vitro stability test for MMAE and brentuximab vedotin, the following results were observed; 1) MMAE was stable in various species plasma, 2) acDrug of brentuximab vedotin was gradually decreased with the incubation time, 3) tAb of brentuximab vedotin was stable in various species plasma, 4) free payload of brentuximab vedotin was gradually increased with the incubation time, 5) the increased amount of free payload was much less than the decreased amount of acDrug. These results suggest that catabolism/metabolism of ADC might play a role in plasma.. Therefore, the search of catabolites/metabolites of the ADC was performed. As results, albumin-conjugated catabolite and the unknown protein-conjugated catabolite were observed and this suggests that the linker-payload was conjugated to the albumin as well as unknown proteins after release from ADC. These results will be very helpful to understand the fate of ADC in the systemic circulation.

Keywords: Brentuximab vedotin, LC-QTOF-MS, Catabolism, Metabolism, ADC

Poster - 77

Drug Interaction Study of CYP3A Modulators and Loxoprofen

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Loxoprofen (LOX) is a non-selective cyclooxygenase inhibitor, widely used for the treatment of pain and inflammation caused by chronic and transitory conditions. Its alcoholic metabolites are formed by carbonyl reductase (CR), and they consist of trans-LOX, which is active, and cis-LOX which is inactive. In addition, LOX can also be converted into an inactive hydroxylated metabolite (OH-LOXs) by cytochrome P450 (CYP). In a previous study, we reported that CYP3A4 is primarily responsible for the formation of OH-LOX in human liver microsomes. Although metabolism by CYP3A4 doesn't produce active metabolite, it can affect the conversion of LOX into trans-/cis-LOX since CYP3A4 activity modulates the substrate LOX concentration. Even though the pharmacokinetics (PK) and metabolism of LOX are well-defined, its CYP-related interactions have not been fully characterized. Therefore, we investigated the metabolism of LOX after pretreatment with dexamethasone (DEX) and ketoconazole (KTC), which induces and inhibit the activities of CYP3A respectively. We monitored their effects on the PK parameters of LOX, cis-LOX and trans-LOX in mice, and demonstrated that their PK parameters significantly changed in presence of DEX and KTC pretreatment. Specifically, DFEX significantly decreased the concentration of the LOX active metabolite formed by CR, which corresponded to an increased concentration of OH-LOX formed by CYP3A4. The opposite result occurred with KTC (a CYP3A inhibitor) pretreatment. Thus, we conclude that concomitant use of LOX with CYP3A modulators may lead to drug-drug interactions and result in minor to severe toxicity even though there is no direct change in the metabolic pathway that forms the LOX active metabolite

Poster - 78

Quantitative and simultaneous analysis of multiple cancer biomarkers using MALDI-TOF based on a parylene-matrix chip

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The parylene-matrix chip was recently developed for the quantitative analysis of molecules smaller than 1 kDa. In this study, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) based on the parylene-matrix chip was performed to clinically diagnose intrahepatic cholangiocarcinoma (IHCC) and colorectal cancer (CRC). The parylene-matrix chip was applied for the detection of small cancer biomarkers including N-methyl-2-pyridone-5-carboxamide (2PY), glutamine, lysophosphatidylcholine (LPC) 16:0, and LPC 18:0. Water-soluble metabolite markers of IHCC, 2PY, and glutamine in serum were extracted using methanol , and water-insoluble metabolite markers of CRC, LPC 16:0, and LPC 18:0 were extracted using Bligh-Dyer method. The feasibility of MALDI-TOF MS based on the parylene-matrix chip was confirmed via analysis of spot-to-spot and shot-to-shot reproducibility. Cancer biomarkers after extraction were quantified using the parylene-matrix chip. For clinical diagnosis of IHCC and CRC, based on cutoff values of 2PY and glutamine, and LPC 16:0 and LPC 18:0, a mixture of biomarkers was analyzed quantitatively using the parylene-matrix chip. Finally, glutamine and LPC 16:0 were simultaneously detected at a range of concentrations in sera from colon cancer patients using the parylene-matrix chip.

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Quantitative carbapenem susceptibility test of carbapenemase-producing enterobacteriaceae using MALDI-TOF based on a parylene-matrix chip

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Carbapenem is the strongest β-lactam antibiotics and acts as inhibitors of the enzymes that catalyze formation of peptidoglycan in the cell wall of bacteria. Recently, the emergence of carbapenem-resistant bacteria seriously threatens this class of lifesaving drugs. Therefore, rapid detection of carbapenemase-producing enterobacteriaceae (CPE) is very important to prevent spread of these strains. Carbapenemase is an important enzyme that are produced by CPE and catalyze the hydrolysis of carbapenem. Typically, MALDI-TOF MS is not appropriate for small molecule analysis because organic matrices make a lot of noise at low m/z range. Parylene-matrix chip was developed for reduce matrix noise, and used to analyze small molecules. Recently, the Parylene-matrix chip has been used for analysis of penicillin-resistant bacteria. The applicability of the Parylene-matrix chip was demonstrated in a quantitative β-lactamase assay that required the quantification of penicillin (*m/z*: $[PEN+H]^+$ = 335.1 and $[PEN+Na]^+$ = 357.8), as well as its hydrolyzed product, penicilloic acid (*m/z* : $[PA+H]^+$ = 353.1). In this study, the Parylene-matrix chip was used in the carbapenemase assay. The assay measured the hydrolysis of 4 carbapenems such as doripenem, ertapenem, imipenem, and meropenem into their hydrolyzed form. Finally, MALDI-TOF MS based carbapenem susceptibility test was carried out with different 60 isolates using Parylene-matrix chip.

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Quantitative analysis of lysophosphatidylcholine in patient serum for diagnosis of sepsis using MALDI-TOF MS based on a parylene-matrix chip

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Lysophosphatidylcholine (LPC) 16:0 has been known to be produced by the action of proinflammatory phospholipase A2 on phosphatidylcholine (PC). LPC is known to stimulate monocytes, macrophages, and T lymphocytes, and it also stimulates neutrophils to destroy ingested bacteria by increasing the production of hydrogen peroxide. In sepsis patients, the deactivation of neutrophils is known to occur, and they are unable to produce adequate hydrogen peroxide to kill bacteria. The concentration of LPC in serum is known to be lower in sepsis patients patients compared with that of healthy controls. Typical diagnosis methods including LC-MS/MS and immunoassays have been reported to have a sensitivity and selectivity in the range of 55–90%. The analysis time has been reported to be >4 min for LC-MS/MS and >2 h for immunoassays.

In this work, MALDI-TOF MS based on a parylene-matrix chip was used for the quantitative analysis of LPC16:0 for medical diagnosis of sepsis. In the first step, MALDI-TOF mass spectra of sepsis patient sera were compared with healthy controls to determine the significantly different mass peaks that can be used as biomarkers of sepsis. The selected mass peaks were identified to represent LPC 16:0 according to LC-MS/MS analysis. Finally, MALDI-TOF MS based on the parylene-matrix chip was applied to the medical diagnosis of sepsis by using patient sera from those with severe sepsis, septic shock, or pneumonia as well as healthy sera.

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Rapid determination of rivaroxaban in rat plasma using liquid-liquid extraction and LC-MRM

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Rivaroxaban (RRN) is the first oral anticoagulant which belongs to direct factor Xa inhibitors. Its success in market has intrigued many researchers to develop various RRN formulations and, as a result, good analytical methods for its determination in biological samples have been demanded. Thus, here, an efficient method to determine RRN in rat plasma using liquid-liquid extraction (LLE) and liquid chromatography and multiple reaction monitoring (LC-MRM) was presented. As the LLE extraction solvent, ethyl acetate was used and it was helpful for the significant reduction of rat plasma volume required for RRN quantitation. The developed method was validated in the aspects of specificity, linearity ($r^2 \geq 0.999$ within 0.5-500 ng/mL), sensitivity (the lower limit of quantitation at 0.5 ng/ mL), accuracy (89.3-107.0%), precision (≤12.7%), recovery (89.2-105.7%), and the stability of RRN in sample extracts under various storage conditions. Also, the validated method was successfully applied to the pharmacokinetic evaluation of RRN after its oral administration to normal rats. To the best of our knowledge, the present method is the first one to employ LLE for extraction and purification of RRN in rat plasma and its efficiency can contribute to the development of new RRN formulations.

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Poster - 82

Qualitative analysis method of methotrexate (MTX) in biological sample by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS)

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Methotrexate (MTX) was formerly known as amethopterin is an antimetabolite and antifolate drug used in treatment of cancer, autoimmune diseases and as an abortifacient in the induction of medical abortions. Methotrexate began to replace the more powerful and toxic antifolate aminopterin and the two should not be confused. Due to this reason, it is important to quantitatively analyze MTX. For this research, we applied to high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) system. Preparation of sample was carried out through the following both preparation method (Exosome kit, ultracentrifuge) with solid-phase extration (SPE). Analysis was performed using Agilent 1200 series HPLC system, Biphenyl columns and using gradient elution, with methanol and 15mM ammonium bicarbonate buffer as mobile phase. The mass spectrometric detection was carried out by triple quadrupole mass spectrometer (A6460, Agilent) equipped with a electro-spray ionization (ESI) interface operating in positive ionization mode using multiple reaction monitoring mode (MRM mode) acquisition for analysis of confirmation.

From this research, we could optimize to analysis MTX 1~5 by LC-MS/MS and detect MTX1 from patient sample. When compared preparation method between exosome kit and ultracentrifuge, both methods was apply to LC-MS/MS method for detection of MTX.

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Effect of Trastuzumab-doxorubicin Conjugates in Cancer Cell line and Identification

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Antibody-drug conjugates (ADCs) are emerging drugs in the field of biopharmaceutical development. ADC are highly targeted biopharmaceutical drug that combine monoclonal antibodies specific to surface antigens present on particular tumor cells with highly potent anti-cancer agents linked via a chemical linker. As of 2019, a total of four new ADCs are on the market, and many big pharma are working with various targets.

We are working on an ADC that targets lung cancer and melanoma. For this purpose, ADC was constructed by linking anti-HER2 antibody Trastuzumab and anticancer drug Doxorubicin with SMCC linker and characterization with MS, UV-Vis and Gel electrophoresis.

In addition, the viability test was performed on the A549 cell line, one of the lung cancer cell lines, and A2028, one of the melanocyte cell line, to confirm the effect. We characterized the trastuzumab-doxorubicin ADC through these experiments. We also concluded that our ADC has a better effect on A549 and A2028 cell line than Trastuzumab alone.

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Comparative Efficiency of ADCs Conjugated with Doxorubicin or Palbociclib on MCF-7 and PC3 cell line and Characterization of ADCs

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The concept of antibody-drug conjugates (ADCs) is based on the ability of a monoclonal antibody to make specific binding, thereby maximizing the killing ability of a cytotoxic agent conjugated with a linker. We constructed the ADCs by linking the anti-cancer drug Doxorubicin or Palbociclib to the anti-EGFR antibody Cetuximab with SMCC linker. In addition, we conducted the viability test on the MCF-7 cell line and PC3 cell line to confirm the effect, and concluded that only cetuximab conjugated with Palbociclib has a better effect on MCF-7 and PC3 cell line than cetuximab alone. Therefore, we utilized Mass Spectrometry to characterize ADCs and determine the cause of this differences.

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Characterization of Antibody-Drug Conjugates Using Middle-up Assay

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Cetuximab is the anti-epidermal-growth-factor-receptor (EGFR) monoclonal antibody for the treatment of colorectal cancer, lung cancer and head and neck cancer. It has a lot of varients resulting from post-translational-modifications (PTMs) including lysine truncation of C-terminal and glycosylation of its four characteristic N-glycosylation sites, which are different from other therapeutic antibodies. These heterogeneity could be observed as multiple peaks in deconvoluted mass spectra that makes it difficult to analyze accurate characters of cetuximab. To reduce heterogeneity of cetuximab, we developed a new middle-up assay. As a result, we were able to characterize cetuximab-conjugated drug with decreased number of peaks in mass spectrometry analysis.

Poster - 86

Surrogate matrix approach for quantitation of endogenous thyroid hormones in rat serum using LC tandem mass spectrometry

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In 2018, OECD guideline for the testing of chemicals was updated to add endocrine-sensitive endpoints for improving detection of potential endocrine activity of test chemicals. The measurements of thyroxine (T_4), triiodothyronine (T_3) were included as part of required endpoints in this revised guideline. Therefore accurate quantitation of endogenous thyroid hormones has become essential for clinical or pre-clinical study regarding new drug development.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is one of the most powerful techniques for absolute quantification because of its sensitivity and selectivity. However, the lack of analyte-free biological matrices is a major obstacle to quantification of endogenous compounds. Four approaches including standard addition, background subtraction, surrogate matrix, and surrogate analyte are commonly utilized to address this drawback.

In this study, surrogate matrix approach using LC-MS/MS was selected for absolute quantification of T_3, T_4 in rat serum and fully validated. Calibration curves for thyroid hormones were made using deionized water as a surrogate matrix. The calibration curves were linear in the range 0.3-50 ng/mL (R^2 = 0.9972) and 3-500 ng/mL (R^2 = 0.9978) for T_3 and T_4 respectively. Inter-batch accuracy (relative error, RE) were < 10 % and inter-batch precision (coefficient of variation, CV) were < 9 %. The recoveries of this method were 78.6-81.2 %, with CVs of 1.2-2.1 %, for T_3, and 71.8-78.2 %, with CVs of 1.4-3.1 %, for T_4 in MQC, HQC. The developed method meets all the requirements of guideline on bioanalytical method validation of KFDA and U.S. FDA. Therefore, this surrogate matrix approach is accurate and precise enough to determine endogenous thyroid hormones in rat serum.

Poster - 87

Differing polyamine levels in the vertex and occipital hair of male and female patients with hair loss

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Hair loss generally begins from the vertex or front of the head and occurs because of increases in androgenic steroid levels. Androgenic steroids are distributed differently in the vertex and occipital regions and are involved in inducing ornithine decarboxylase expression. Therefore, the distribution of polyamines may be altered in different scalp regions. This study was conducted to evaluate the pathogenesis and diagnosis of patients with hair loss. For the overall metabolic profiling of polyamines in patients with hair loss, a liquid chromatography-mass spectrometry-based quantitative profiling method was used. We investigated the differences in polyamine levels in different regions of the hair between patients with male-pattern baldness and those with female-pattern hair loss. The levels of most polyamines were higher in the vertex region than in the occipital region, and *N*-acetyl polyamine levels significantly differed. We verified that the distribution of polyamines differed in various regions of the head. Because of the different polyamine concentrations in the vertex and occipital regions, both male- and female-pattern baldness were affected by ornithine decarboxylase and spermidine/spermine N^1-acetyltransferase.

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Validated LC-MS/MS method for quantification of rosuvastatin in small volume of human blood

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Volumetric absorptive microsampling (VAMS), developed as a microsampling collection device, can simplify the collection of an accurate volume of blood regardless of volumetric hematocrit level. The purpose of this study was to develop a simple and rapid liquid chromatography tandem-mass spectrometry (LC-MS/MS) method for quantitation of rosuvastatin collected on VAMS. Quantitative determination of rosuvastatin was carried out in 10 μL of human blood by LC-MS/MS. Linear correlation coefficients (r) were higher than 0.999 at concentration levels of 1-100 ng/mL. The response of analyte was more than 5 times that of the blank at lower limit of quantitation (LLOQ, 1 ng/mL) and the retention time of analyte was 0.97 min. The standard deviations between the measured and theoretical values for the six standard concentrations showed that they were within the acceptance criteria (±15% of nominal concentrations, except ±20% at LLOQ). In the stability test, rosuvastatin collected by VAMS device was stable at storage conditions of bench top, autosampler, 3 cycles of freeze and thaw, after short-term and long-term storage time. This study demonstrates that this LC-MS/MS method is reproducible and stable with adequate sensitivity in a small amount of blood (10 μL). The fully validated LC-MS/MS method may be applied to clinical trials such as bioequivalence and pharmacokinetic studies of rosuvastatin.

Poster - 89

Nanostructured TiO_2 materials for the analysis of gout-related crystals using LDI-ToF mass spectrometry

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The crystals of monosodium urate monohydrate (MSU) and calcium pyrophosphate dihydrate (CPPD) have been known to induce gout and pseudogout, respectively, which are deposited in joints or tissues to cause severe pain. TiO_2 nanostructures including TiO_2 nanowires synthesized from wet-corrosion method, TiO_2 nanoparticles (P25) and the mixture of of TiO_2 nanowires / P25 were presented as solid matrices for the medical diagnosis of gout and pseudo-gout using laser desorption/ionization mass spectrometry (LDI-MS). As the first step, the suitable TiO_2 nanostructured materials were selected as solid matrices for the quantitative as well as qualitative analysis of MSU and CPPD crystals by using three kinds of TiO_2 nanostructures. The feasibility of LDI-ToF MS based the selected TiO2 nanostructures was tested by using spiked synovial fluids. The analysis results of MSU and CPPD showed that (1) the ionization reaction of solid matrices should be optimized by considering electrostatic interaction between analyte and solid matrices, and (2) MSU and CPPD crystals in synovial fluid could be analyzed for the medical diagnosis of pseudo-gout by using LDI-ToF mass spectrum with the mixture of TiO_2 nanowires / P25 as a solid matrix.

Keywords: MSU, CPPD, Gout, Pseudogout, LDI-ToF, Mass spectroscopy

Poster - 90

Analysis of N-Linked Glycan of Intact Phospholipase A2 from Honeybee Venom by Mass Spectrometry

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Bee venom contains a complex mixture of biologically active agents, such as peptides, enzymes, biogenic amines. The main components of honeybee (Apis mellifera) venom are the enzymes phospholipase A2 and hyaluronidase, and the low-molecular-weight proteins melittin and apamine. Characterization of the oligosaccharide structures on PLA has been reported and fourteen different structures of heterogeneous N-glycans from honeybee venom PLA have been elucidated by HPLC in combination with composition analysis, methylation analysis and NMR spectroscopy (Habermann E et al., 1972). In this study, we analyzed PLA2 purified from honeybee venom using LC chromatography and Mass spectrometry.LC-MS analysis was performed using an Agilent 1290 Infinity LC (Agilent, UK) coupled to a 6530 O-TOF Mass spectrometer (Agilent, UK). A voume equivalent to 5 μg of sample preparation was injected on an XBridge BEH300, C8, 5 μm, 2.1 × 150 mm column (Agilent, UK) set at 80 °C. The gradient was generated at a flow rate of 1.0 ml/min using 0.1% TFA for mobile phase A and acetonitrile containing 0.08% TFA for mobile phase B. B was raised from 20 to 50% in 30 min followed by a 2 min washing step at 90% B and a 25-min re-equilibration period. The results indicate that PLA2 is glycosylated and non-glycosylated at a constant rate. The glycans also had different numbers of mannose.

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Analysis of galactose by MALDI-ToF mass spectrometry using TiO_2 nanowire chip

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New quantification method of galactose was presented for the newborn screening test of galactosemia by using MALDI-ToF mass spectrometry (MS) using TiO_2 nanowire chip. Analysis of galactosemia, an inborn metabolic disease, is generally performed with various detection methods. For the quantitative analysis of galactose, reduction potential of galactose was used to oxidize o-phenylene diamine (OPD) into 2,3-diaminophenazin (DA) which could be quantitatively analyzed by MALDI-ToF MS based on TiO_2 nanowire chip. TiO_2 nanowire chip was developed for the qualitative and quantitative analysis of galactose in PBS buffer, serum and dried blood spot (DBS) using MALDI-ToF MS by reducing the noise of conventional organic matrix. TiO_2 nanowire chip was synthesized from wet-corrosion method. To demonstrate the feasibility of this method, interference of glucose and other proteins in serum was analyzed. Concentration of galactose in PCB buffer, serum and DBS and the intensity of mass peak of OPD and DA were linearly correlated for the application to the newborn screening test of galactosemia.

Keywords: MALDI-ToF mass spectrometry, TiO_2 nanowire chip, galactose, galactosemia, o-phenylene diamine

Poster - 92

Novel GlcNAc-containing oligosaccharides in Aspergillus oryzae β-galactosidase-treated bovine whey permeate

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Bovine milk oligosaccharides (BMOs) in whey permeate resemble human milk oligosaccharides (HMOs), indicating that whey permeate is a potential source for milk oligosaccharides that carry HMO bioactivities. However, the recovery of oligosaccharides from whey permeate has been hindered by the low abundance of target oligosaccharides and the high concentration of undesirable lactose molecules, which overshadow the biological activity of the oligosaccharides. Lactose was hydrolyzed by Aspergillus oryzae β-galactosidase to selectively enrich the bioactive oligosaccharides through membrane separation. The generated monosaccharides were much smaller and easily separated from BMOs. High-resolution mass spectrometry analyses revealed that β-linkage-containing BMOs were degraded and that new oligosaccharides were produced during the enzymatic reaction. The synthesized oligosaccharides have N-acetylglucosamine (GlcNAc) at the reducing ends, and their degree of polymerization ranges from 5 to 11. The produced hetero-oligosaccharides could be used as the next generation of bio-therapeutic oligosaccharides and are capable of establishing a healthy intestinal microbial balance.

Poster - 93

Metabolomic assessment of early-onset of preeclampsia

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Preeclampsia (PE) is a hypertensive disease associated with pregnancy that has not yet been clearly elucidated. The disorder may cause fetal growth retardation, premature birth, and proteinuria. If it progresses seriously, it will lead to complications such as pheochromocytoma, chronic hypertension, and death. Thus, special care is required. Often, preeclampsia is similar to normal pregnancy symptoms such as weight gain, swelling, and headache. And some pregnant women have no symptoms at all; therefore, it is important to properly diagnose preeclampsia through periodic antenatal screening and early diagnosis. In fact, there are some studies on biomarker discovery using various analytical platforms to measure various molecular species such as amino acids, fatty acids, and organic acids for preeclamptic patients. However, little is known about overall phospholipid profile of blood samples.

In this study, metabolic profiling of maternal plasma samples in mid-trimester was performed using gas chromatography-time of flight mass spectrometry (GC-TOF MS) and liquid chromatography orbitrap mass spectrometry (LC-Orbitrap MS). As a result, a total of 329 metabolites were identified. First, partial least squares-discriminant analysis (PLS-DA) showed a clear discrimination between preeclampsia and healthy control groups. Since then, binary logistic regression analysis has suggested a metabolic biomarker model (5 metabolites). This prediction model successfully discriminated between the preeclampsia group and the healthy control group in the middle of pregnancy.

Poster - 94

Combi-matrix of CHCA and graphene for detection of L-Thyroxine (T_4)

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Combi-matrix of CHCA and graphene is introduced as a new type of solid matrix in MALDI-TOF mass spectrometry for improved detection of T_4 (L-Thyroxine). T_4 is a hormone secreted from thymus and it is related to congenital hypothyroidism. Cut-off value of medical diagnosis of congenital hypothyroidism is known to be around 50 ng/mL. For the quantitative detection of T_4 at this concentration range, combi-matrix was composed of the conventional organic matrix of CHCA and solid matrix of graphene, which can effectively absorb the laser radiation. Ionization efficiency of combi-matrix were analyzed and the quantitative mass analysis of T_4 standard sample and T_4-spiked serum was carried out. Optical and thermal properties of combi-matrix were characterized by UV-VIS spectrometer and differential scanning calorimetry to show the changes in heat capacity and enthalpy. Based on such properties, mixed-component matrix results in enhanced signal-to-noise ratio, lower limits of detection and linear detection response of T4 compared to the conventional matrix in MALDI-TOF mass spectrometric analysis. MALDI-TOF mass spectrometry using combi-matrix presents the improved detection of T_4 and demonstrates the applicability to clinical diagnosis of congenital hypothyroidism.

Keywords: MALDI-ToF mass spectrometry, Combi-matrix, graphene, T_4, Congenital hypothyroidism

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Breath Analysis with Thermal Desorption GCMS

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Various gas detection methods have been applied to find a non-invasive diagnostic method with volatile organic compounds (VOCs) of human effluents. The exhaled breath is one of the most easily collectable human samples including profound information related to various human diseases. To show the feasibility of the diagnostic method with VOC markers in breath, exhaled breath samples of uremic patients were collected before and after hemodialysis. VOCs were concentrated with thermal desorption tubes or solid phase micro extraction (SPME) sampling fibers and then desorbed and detected with GCMS. Numerous exhaled VOCs were detected from 2 uremic patients at pre- and after hemo-dialysis. After the dialysis, concentration of dimethyl sulfide among volatile sulphur compounds and acetone among ketones were decreased, but concentration of isoprene, a possible by-product of cholesterol metabolism, rubber component or contaminants, was increased in uremic patient. Also, several relevant uremic toxins were detected. The GCMS of human exhaled VOCs may provide useful information on dialysis perfomace and/or characterization of the dialysis patients with a simple breath sample.

Poster - 96

Simple determination method for bioactive compounds of Bojungikgi tang by UPLC-MS/MS: Application to clinical pharmacokinetics

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Bojungikgi-tang (BJIGT) is a traditional medicine consisted of 8 herbs to treat digestive diseases. It was formulated as a soft-extract to improve patient compliance, and it is currently covered by Korean national health insurance. The aim of this study was to develop and validate determination methods for quality control bioactive compounds and their metabolites of BJIGT soft-extract such as glycyrrhizin (GL), 18β-glycyrrhetinic acid (GLA), ginsenoside Rb1 (Rb1), ginsenoside Rg1 (Rg1), hesperidin (HD) and hesperetin (HT) in human plasma. The ultra-performance liquid chromatography (UPLC) coupled with electrospray ionization tamdem mass spectrometery methods were developed to evaluate the pharmacokinetics of these compounds. The chromatography separation was performed on C18 or biphenyl column with gradient mobile phase consisting of water (containg 0.1% formic acid or 2 mM ammomium acetate), methanol or acetonitrile (containg 0.1% formic acid or not). The analytes were detected using MRM mode with positive or negative electrospray ionization, and they were pretreated by either protein precipitation or liquid-liquid extraction.

The callibration curves were fitted over the range of 2-500 ng/mL for GL and GLA, 0.2-50 ng/mL for Rb1 and Rg1, and 0.1-10 ng/mL for HD and HT with correlation coefficients greater than 0.991. Coefficients of variation (CVs) of inter- and intra batch precision and accuracy for six compounds were within ±15% (within ±20% for LLOQ). Therefore, the developed methods satisfied the ICH and FDA guidelines and were successfully applied to evaluate the pharmacokinetics of bioactive compounds of BJIGT soft-extract after oral administration to healthy male Korean subjects.

Poster - 97

A middle-up strategy for glycosylation quality assessment of monoclonal antibodies by UPLC/MS

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Glycosylation on monoclonal antibodies(mAbs) is considered as one of important attributes that can affect quality, safety, and potency of drugs. Therefore, glycosylation should be characterized from the early drug development to final lot release. Recently, intact protein analysis has been widely used technique to monitor glycosylation on mAbs in large-scale analysis. However, dimeric glycoproteoform and other PTMs of mAbs make data interpretation difficult in intact level. Here, we developed a middle-up approach by reducing sample complexity through enzymatic cleavage and chemical reduction to achieve fast, sensitive, and overall analysis of glycosylation on mAbs. IdeS, the hinge region cleaveage enzyme, is used to yield two Fc/2 and the dimeric Fab subunits. DTT was added to reduce disulfide bonds and generate two subunits(heavy and light chain). Additionally, each mAb was treated with combination of IdeS and DTT to release three subunits(light chain, Fc/2 and Fd domain). Finally these subunits were analyzed by on-line desalting and diphenyl-UPLC/q-TOF MS. Subunit–specific glycan patterns and C-terminal lysine clipping were identified. Interestingly, glycan profiling obtained respectively from released and subunit analysis represented high correlation. Our approach can be a benefit in the quality control of antibody-based biologics, and the assessment of similarity between biosimilars.

Poster - 98

Development of mass spectrometry-based receptor tyrosine kinase high throughput screening platform technology for the identification of biomarkers and for the cancer therapy

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Receptor tyrosine kinases (RTKs) play key roles in cellular signal transduction, cell cycle regulation, cell division, and cell differentiation. Dysregulation of RTK-activated pathways, often by receptor overexpression, gene amplification, or genetic mutation, is a causal factor underlying numerous cancers. In this study, we have developed a multiple reaction monitoring-based assay for quantitative profiling of 54 RTKs. This assay detects 56 stable isotope internal standard (SIS) from 54 receptor tyrosine kinases in a single run. Quantitative comparisons were based on the labeled reference peptide method. We implemented this assay for the two human cololectal cancer cell lines, which are SW480 and SW480/Beva (Bevacizumab resistance cell lines). We observed distinct RTK expression changes in SW480 and SW480/Beva cell lines by using this assay. Shotgun analysis and western blot analysis data were highly consistent with multiple reaction monitoring data. EGFR was significantly increased in SW480/Beva cell lines, which was compared to the EGFR expression in SW480 cell lines. Migration efficacy for the SW480/Beva cell lines was significantly increased, which was compared to the migration efficacy for the SW480 cell lines. EGFR inhibitors significantly suppressed migration efficacy for the SW480/Beva cell lines. In conclusion, this assay is expected to the useful experimental tool for the identification of biomarkers and for the cancer therpy.

Poster - 99

Studies on human epidermal growth factor receptor 2/4 (Her2/4) inhibitors that cause changes in protein expression level of protozoan parasite, *Toxoplasma gondii*

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***Toxoplasma gondii*, a ubiquitous, intracellular parasite of the phylum Apicomplexa, infects an estimated one-third of the human population as well as a broad range of warm-blooded animals. We have observed in previous reports that some tyrosine kinase inhibitors suppressed the growth of *T. gondii* infected with host ARPE-10 cells. These results suggested that inhibitors of receptor tyrosine kinase such as HER2/4 may be used as a therapeutic agent for inhibiting parasite growth with minimal adverse effects on host cells. In this report, we conducted a proteomic analysis to observe changes in host proteins that were altered via infection with *T. gondii* and the treatment of HER2/4 inhibitors. As a result, the expression level of PTPK, SEMA7A, PPP2R2A, RUS1 and GOLM1 protein were significantly changed, and the results were confirmed by western blot analysis. Changes in various ost proteins, including these five proteins, can be used as a basis for explaining the effects of T. gondii infections and HER2/4 inhibitors.**

Poster - 100

Changes in the proteomic profiles of mouse brain after infection with Toxoplasma gondii

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Toxoplasma gondii is an opportunistic protozoan that causes cosmopolitan zoonosis toxoplasmosis, mainly through oral infections, blood infections and diaplacental infections. Approximately 30% of the human population worldwide are chronically infected with *T. gondii*. To better understand what this parasite does to human brains, we performed a comprehensive systems analysis of the infected brain by a micro-scale basic reverse phase liquid chromatographic (bRPLC) fractionation method. In the present study, we explored the proteomic profiles of brain tissues of the specific pathogen-free Balb/c mice at 2 weeks, 4 weeks and 8 weeks after infection with cysts of the *Toxoplasma gondii RH* strain. We identified 3,137 proteins out of which 1,221 proteins were differentially expressed (≥1.5-fold). Gene Ontology analysis showed that these proteins were mainly involved in cell morphology, cell to cell signaling interaction and cell death and survival, and will be beneficial for the understanding of molecular mechanisms of *T. gondii* pathogenesis. Functional characterization of the altered proteins may enhance understanding of the host responses to *T. gondii* infection and lead to the identification of new therapeutic targets. These results provided an insight into the responsive relationship between *T. gondii* and the host brain tissues, which will shed light on our understanding of the mechanisms of pathogenesis in toxoplasmic encephalitis, and facilitate the discovery of new methods of diagnosis, prevention, control and treatment of toxoplasmic encephalopathy.

Poster - 101

Effect of therapeutic agent BS11 on interaction between intestinal microbes and metabolic responses of metabolic diseases

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Obesity and diabetes are global health problems associated with metabolic diseases. BS11 is a traditional Korean and Chinese herbal medicine and widely used as a treatment for obesity-related disease. In this study, we applied liquid chromatography mass spectrometry-based lipidomics to investigate the effect of BS11 on LDL knockout mice fed western diet (WD, 40% fat). In addition, we also observed changes in the metabolites of 10 short chain fatty acids and 22 bile acids that are relevant to the intestinal microbiome. Statistical analysis from the identified 827 lipids showed a unique lipid profile after treatment. Furthermore, the integrative analysis of lipids, metabolites and microbiota demonstrated that BS11 regulates hepatic lipid metabolism and immune response associated with the gut microbiom. Consequently, we have verified the lipids and microbiota linkage-based functional properties of BS11 and suggested that putative therapeutic effects.

Keywords : Lipidomics, Gut microbiome, Metabolic diseases, Mass spectrometry, and Herbal medicine, Metabolomics

Poster - 102

A sensitive LC-MRM method to determine montelukast in rat plasma using liquid-liquid extraction

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Montelukast (ML), an antagonist of CysLT1, is widely used to relieve the symptoms of rhinitis and asthma. However, due to its chemical instability, its dosage forms have been limited to solid state ones, and, recently, the development of its variations, such as syrup, has been actively attempted. Therefore, as a part of these efforts, a sensitive method to determine ML in rat plasma, a prerequisite for the successful development of its new dosage forms, was presented here. For efficient extraction and purification of ML in rat plasma sample, liquid-liquid extraction (LLE) with ethylacetate and dichloromethane mixture was employed, and the extracted solution was analyzed through an LC-MRM assay. The developed method was successfully validated following the FDA guidelines in the aspects of selectivity, limit of quantification (0.5 ng/mL), linearity ($r^2 \geq 0.998$ within 0.5 to 500 ng/mL), matrix effect (-14.83 to -6.91%), accuracy (88.37 to 100.64%), precision (2.99 to 13.25 %), recovery (80.78 to 86.34%), and stability (85.84 to 102.54%). Additionally, from the comparisonal experiments, it was confirmed that the present method is less vulnerable to the instrument contamination than the previously-reported method is. Finally, the present method was successfully applied to pharmacokinetic evaluation of Singulair® and a ML syrup under development. To the best of our knowledge, the present method is the first one to employ LLE with ethylacetate and dichloromethane mixture for extraction and purification of ML in rat plasma and its efficiency could contribute to the development of new ML dosage forms.

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Poster - 103

Comparative proteome analysis for three different developmental stage of muscle cell

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The degenerative loss of skeletal muscle mass (0.5–1% loss per year after the age of 50), quality, and strength associated with aging is called as sarcopenia. Decrease of muscle mass induces both muscle and bone weakness resulting decrease of various metabolism. However, exact mechanism of sarcopenia still is unclear. Clinically, sarcopenia has been diagnosed by physical examination, dual-energy X-ray absorptiometry (DEXA), and computed tomography (CT) scan. However, some of examination have limitation for exact diagnosis of sarcopenia. Therefore, there have been huge clnical unmet needs for molecular diagnosis using liqud biopsy sample. Herein, global proteome from three different developmental stage of muscle cell (myblast, myocyte and myotubule) were compared using high resolution mass spectrometry coupled with liquid chromatography in label free quantitation way in order to investigate mechanism of muscle development and, also to discover muscle-regeneration factors. Totally, 5,819 proteins were quantified, and gene ontology analysis revealed that positive regulation interleukin secretion and negative regulation of macrophage chemotaxis were statistically enriched in muscle development. In addition, some candidate proteins were identified through a special pathway and disease & funtion associated with Ingenuity Patyway Analysis (IPA). Further investigation will be followed by integration of mouse muscle proteome analysis from sarcopenia model.

Poster - 104

Simultaneous Determination of the five components in Citrus junos Using a UPLC–PDA and UPLC/ESI-MS.

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Citrus junos seed has been traditionally used for the treatment of cancer and neuralgia and known for using the edible oil, cosmetic perfume. In this study, an ultra-performance liquid chromatography–photodiode array (UPLC–PDA) method to evaluate was developed and then used for the simultaneous quantitative analysis of five components in *C. junos*. The Waters Acquity UPLC HSS T3 Column C18 (2.1 x 100 mm, 1.8 μm) was used for this separation, maintained at 40°C. The mobile phase used was distilled water and acetonitrile with gradient elution. To identify quantity of five components, we used a mass spectrometer (MS) with an electrospray ionization (ESI) source. The regression equation showed great linearity: correlation coefficients were ≥ 0.9996. The limits of detection (LOD) and limits of quantification (LOQ) of the five compounds were 0.02 – 0.04 and 0.06 – 0.14 μg/mL, respectively. The recoveries of extraction were ranged from 97.51 to 108.67%. The relative standard deviation (RSD) values of intra- and inter-day precision were respectively 0.06 – 1.55 and 0.09 – 1.68%. The UPLC–PDA method was validated to simultaneously analyze the quantity of five components in *C. junos* in this study.

Poster - 105

Proteomics analysis of thyroid tissues revealed the change of aminoacyl-tRNA synthetases and its interacting proteins in follicular thyroid carcinoma and follicular adenoma

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Aminoacyl tRNA synthetases (ARSs) have a canonical function in charging a specific cognate amino acid on one of all its compatible cognate tRNAs to form an aminoacyl-tRNA. Besides this classical function, ARSs and ARS-interacting multifunctional proteins (AIMPs) have been investigated as key proteins in many diseases including inflammation, tumor with non-canonical functions. Focusing on its relationships with diseases, we have investigated the possibility of ARSs AIMPs as surrogate biomarkers to diagnose and to monitor specific diseases. In this study, 23 of ARSs/AIMPs were identified and quantified with label free quantitation from global proteome analysis of thyroid tissues from follicular thyroid carcinoma (FTC) patients and follicular adenocarcinoma (FA) patients. Further, we developed sample preparation method to extract and measure the relative abundance of ARSs and its interacting proteins from FFPE (formalin Fixed Paraffin Embedded) sample with mass spectrometry. As a result of comparative analysis from DIA analysis, AIMP1, WARS1 and YARS1 were increase in FTC tissues compared to FA tissues. Identical result was obtained from quantitative proteome analysis of formaldehyde-fixed paraffine embedded (FFPE) thyroid tissue. This result can be applied to develop any kind of diagnosis kit to classify FTC patients from FA patients without surgery.

Poster - 106

Multi-Attribute Analysis of Monoclonal Antibodies Using the Agilent InfinityLab 2D-LC Solution and Q-TOF MS

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In recent years, two-deminsional liquid chromatography (2D-LC) has been shown to be highly promising for the detailed characterization of monoclonal antibodies(mAbs). This application note describes the use of the Agilent 1290 Infinity II 2D-LC System and the Agilent 6530 Q-TOF LC/MS for multi-attribute analysis directly from cell culture supernatants. The multi-attribute analysis directly from cell culture supernatants. The multi-attribute analyzer combines protein A affinity chromatography with size exclusion chromatography (SEC) and liquid chromatography/mass spectrometry (LC/MS) in a (multiple) heart-cutting three-dimensional (3D) setup. This workflow enables simultaneous assessment of mAb titer, size variants, molecular weight (mol wt), amino acid sequence, and post-translational modifications.

Poster - 107

Enhanced analysis of veterinary drug in livestock products with novel LC-MS ionization source

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These days regulatory agency is tightening veterinary medicine regulation in livestock products for the purpose of ensuring food safety, like antibiotics, pharmaceutical, hormones. Also, trace level analysis in complex matrices should generate the information required to meet the regulation. As international regulations, Maximum residue limits (MRL) for these compounds can vary worldwide, but are generally in the low concentration level, the need for multi-analyte screening procedures to efficiently detect violating residues is ever increasing. Analysts must faced up to an unexpected difficulty as the complexity of sample matrices and differing physicochemical characteristics of contaminating compounds thus, requiring reproducibility of work, to ensure all residues of interest that could be targeted.

This report shows novel LC-MS ionization techniques, UniSpray, and comparision between comparative evaluation of a legacy Tandem Quadrupole mass spectrometer (ESI) with a new source on analysis of 103 veterinary medicine is also summarized. UniSpray demonstrated sensitivities were increased while extending the scope of multi-class analytes screened to excelled levels of detection in a single workflow.

Poster - 108

Multi-residue Analysis of 43 Pesticides in Fishery Products Using Gas Chromatography with Tandem Mass Spectrometry (GC-MS/MS)

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A multi-residue analytical method was developed and validated to investigate pesticide residues in fishery products. In this study, we developed analytical method for such as organochlorine, organophosphorus pesticides and so on. The target analytes are 49 pesticides and their metabolites. The analytes are extracted from muscle tissue of fish with high speed dispersion in ethyl acetate followed by solvent exchange to acetonitrile and clean-up using low temperature freezing, centrifugation, and dispersive solid phase extraction. Pesticide residues were determined by using GC-MS/MS. The linearity, accuracy, and precision at three concentrations (0.01, 0.02, and 0.1 mg/kg) were evaluated by Codex guidelines (CAC/GL 40-1993). As a result, 43 pesticides showed suitable results to be applied to the screening method. Although analytical method was available for most pesticides, simultaneously analysis was not possible for 6 compounds. Forty of the 43 pesticides were satisfied with the accuracy, precision and linearity in the guidelines and can be applied to the quantitative method. Therefore, this method is suitable for determining the 43 pesticides in fishery products.

Key word: Fishery products, Pesticide, GC-MS/MS, Analysis

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Poster - 109

Multi-Residue Determination of 83 Veterinary Drugs in Fishery Products by LC–MS/MS

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Multi-residue analytical method has been developed and validated for 83 veterinary drugs in fishery products based on LC-MS/MS. The target analytes included antibiotics, anthelmintics and anti-inflammatory drugs. The target analytes were extracted from fishery samples by using 80% acetonitrile, and then clean-up with C_{18} and acetonitrile saturated with n-hexane. The developed method was validated according to the Codex guideline (CAC/GL 71-2009). As a results, recoveries of the veterinary drugs ranged from 63.2 to 120%. At all target concentrations, within-laboratory CV was < 30%, and the lowest limits of detection and quantification were in the range between 0.02 and 12 μg/kg. The developed method was satisfied requirements of the codex guideline with high accuracy, precision and acceptable sensitivity. Therefore, the analytical method was demonstrated to be simple, economical and reliable for the fast monitoring of veterinary drug residues in fishery products.

Key words: Multi-residue, Fishery products, Veterinary, LC-MS/MS

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Poster - 110

Multi-Residue Determination of 81 Veterinary Drug Residues in Livestock Products using LC-MS/MS

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The aim of the present study was to develop a multi-residue quantitative method for the analysis of 81 veterinary drugs including antibiotics, anthelmintics and anti-inflammatory drugs in livestock products using liquid chromatography-tandem mass spectrometry.

In this study, five representative livestock products (beef, pork, chicken, milk, egg) were spiking (0.5×MRL, 1×MRL, 2×MRL or 1×LOQ, 2×LOQ, 10×LOQ) on the sample according to the MRL (maximum residual limit) and LOQ (limit of quantification). The extraction solvents was 80% acetonitrile, and then clean-up with C_{18}, and filtered with PTFE syringe filter based on FSIS sample preparation method (CLG-MRM 1.08). The analytes were qualified and quantified by LC-MS/MS in the positive/negative ion mode using multiple reaction monitoring (MRM). The coefficient of determination(r^2) was more than 0.98 at matrix-matched calibration curves. The recoveries ranged from 60 – 120%, CV was < 30 % corresponding to the CODEX guideline (CAC/GL-71-2009). Therefore, this method is acceptable for screening and quantification of 81 veterinary drugs in livestock products.

Poster - 111

Analysis of Sorbitol of Brown Rice and White Rice in Storage Using a Mass Spectrometer

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Food adulteration about crops is a serious problem with harvest season of each year. For example, some products of rice in the market were mixed with long-term stored one because of expensive selling price of new rice. It is heavy damage to both rice farmers and consumers.

Currently, method by guaiacol, oxydol, p-phenylenediamine is widely used for measurement of the freshness of rice. However, this method has limitation to distinguish new and stale rice. Therefore, new technology with reliability and accuracy is strongly required.

According to our previous study, it was confirmed that the content of sorbitol in rice, which is a sugar alcohol is gradually increased after harvesting the rice. Sorbitol was extracted from the rice sample by several steps containing weighing the rice, heating, shaking and centrifugation. After extraction process, content of sorbitol from each sample was analyzed by a mass spectrometer. Herein, we applied this method to analyze brown and white rice.

All of 14 samples in this study were produced in 2018 and stored at room temperature. After these were peeled off the husk, brown and white rice samples were obtained for sorbitol analysis. Each rice sample has been analyzed monthly during six months. The content of sorbitol was 7.9-10.5 mg/kg in brown rice, and 2.5-4.3 mg/kg in white rice.

Conclusionally, we confirmed the difference of the content of sorbitol between brown and white rice. Based on the results of this study, we will more improve this method in order to apply to various grains.

Poster - 112

Selection and optimization of the identification marker in gamma-irradiated soybeans for a HS-SPME-GC-MS analysis

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The aim of this study was to explore the identification marker for gamma-irradiated soybeans and to optimize the extraction conditions for the selected marker using gas chromatography-mass spectrometry coupled with a headspace solid-phase microextraction technique (HS-SPME-GC-MS). From the partial least squares-discriminant analysis of chromatograms obtained from non-irradiated and irradiated soybean samples (0.5 – 5 kGy), 1,7-hexadecadiene was tentatively selected as the identification marker. Optimal HS-SPME extraction conditions were determined as a CAR/PDMS fiber for a solid phase, 98°C for extraction temperature, and 55 min for extraction time through the RSM experiments for the standard material spiked in the non-irradiated soybeans. As an application test, 1,7-hexadecadiene was detected in all soybean samples irradiated at > 0.1 kGy under the optimized HS-SPME-GC-MS conditions except for non-irradiated samples. This result implies that all irradiated samples tested were correctly identified as irradiated using the identification marker under the optimized HS-SPME-GC-MS conditions.

Poster - 113

Simultaneous determination of B-group vitamins in health functional foods by LC-MS/MS

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Vitamin B is a group of water-soluble vitamins that play important roles in cell metabolism. The absence of individual B vitamins in a diet can lead to several conditions including depression and high blood pressure so they are often added to foods. Analysis of food samples can be challenging, as the matrices are complex and sensitive methods typically require highly selective sample clean up procedures.

The purpose of this study was to optimize chromatographic and detection conditions for the simultaneous determination of vitamin B in health functional foods. The method was designed using liquid chromatography-tandem mass spectrometry(LC-MS/MS). Electrospray ionization(ESI) in multiple reaction monitoring(MRM) mode was performed in positive ion mode. The analyzed vitamins included thiamine, riboflavin, nicotinic acid, nicotinamide, pyridoxine, biotin, pantothenic acid, folic acid and cyanocobalamin. The method utilized a reversed phase C_{18} column (3 ㎛, 2.0×100 ㎜) with a gradient mobile elution profile, performed at a flow rate of 0.3 ㎖/min.

The method was validated for selectivity, linearity, the limit of detection(LOD), the lmit of quantitation(LOQ), precision and accuracy. It was proved to be suitable for routine quantitative analysis of commercially available multi-vitamin products.

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Poster - 114

Comprehensive metabolomic analysis of sesame seeds

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Sesames are enriched with amino acid, essential fatty acids, carbohydrates, hydroxy acid. The nutritional quality and metabolic uniqueness of sesame is determined by a range of environmental factors (e.g. climate and soil) as well as genetic factors.

Therefore, we metabo-typed sesame seeds cultivated in East Asia (Korea and China), South Asia (India and Pakistan), and Africa (Nigeria and Ethiopia). A total of 243 primary and secondary metabolites were profiled using gas-chromatography time-of-flight mass spectrometry (GC-TOF MS) and liquid-chromatography Orbitrap mass spectrometry (LC-Orbitrap MS).

As a result, the PLS-DA model was able to confirm that the lines of sesame seeds in Korea, China and other countries were different. Based on this model, when selecting seven major metabolites and doing ROC analysis, it was confirmed that the AUC value was very well predicted at 0.913-0.999 (Glycerol, Scopoletin, Thiamine, DL-Carnitine, D-Raffinose, 1-monopalmitin). In addition, compared with other countries, the amino acid group of Korean sesame was abundant and the similar patterns among regions were identified as *PatternHunter* module in *MetaboAnalyst.*

Subsequent exploration of geographical characteristics combined with localized metabolic characteristics suggested a presumed relevance that could be generalized and applied to genuine decisions on the domestic cultivation source of general agricultural products.

Poster - 115

Analysis of pyrrolizidine alkaloids and their N-oxides in plants using LC-MS/MS with low pressure column switching

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Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by many plant species. Exposure to pyrrolizidine alkaloids in food, in particular for frequent and high consumers of tea and herbal infusions, is a possible long-term concern for human health due to their potential carcinogenicity.

To detect the toxic compounds in plant material, we developed an automated online-SPE UHPLC-MS/MS method which simplified laborious sample preparation and overcame the limitation of low pressure SPE column. While the analytes were retained on the SPE column, the sample matrix were washed out by the loading solvent. Following the valve switching, the analytes were eluted from the SPE column to the additional loop system. After trapping the analytes in the loop system, there were transferred to analytical column.

The proposed method was applied to analyse 30 PAs and their related N-Oxide in various tea samples. All calibration curves presented correlation coefficients (r^2) greater than 0.99 with S/N >10 for LLOQ Levels. And we were able to quantify the PAs in the range of 10 to 400 μg/kg.

In this work we report an online-SPE method for high-sensitivity analysis was successfully developed for PAs analysis in plant material.

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Development and validation of UPLC-MS/MS method for analysis of emodin in tartary buckwheat flower

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Emodin is an anthraquinone present in buckwheat and has been known to have anti-tumor, anti-inflammatory and laxative effects. However, emodin content in buckwheat is very low; therefore, it is limitedly detected by HPLC. The objective of this study was to develop and validate a sensitive UPLC-MS/MS method for analysis of emodin in methanol extract of tartary buckwheat flower (TBF), in which emodin is expected to be the most abundant among the parts of buckwheat. Chromatographic analysis was performed using UPLC-ESI-MS/MS (Acquity UPLC H class and Xevo TQD) with HSS T3 column (100 mm × 2.1 mm, 1.8 μm). An MRM mode ([M-H]-, m/z 269.1→225.1) was operated for the detection and quantification of emodin. Method validation was performed by linearity, limit of detection (LOD), limit of quantification (LOQ), and precision. The calibration curves showed a good linearity over the concentration range of 0.078 – 5 μg/mL and the determination coefficient (R^2) was 0.999. The LOD and LOQ were 0.37 and 1.1 μg/mL, respectively. Recovery rate and relative standard deviation were 98.89% and 1.23%, respectively. Emodin content in TBF was 26.2 ± 1.4 μg/g (dry basis). The results might be useful for analysis of emodin contents in the dietary buckwheat products.

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MRM/MS-based Platform for Quantitative Determination of Sialic Acids in Human and Mammal milk

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Sialic acids conjugated in carbohydrates, lipids, and protein are contributed to play the valuable functions such as immature immune system, brain development, and intestinal health. Sialic acids commonly found in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) respectively. However, Neu5Gc which cannot synthesize in human could lead to an immune response interacting with Neu5Gc specific-antibodies in human. Therefore, it is important to determine and quantify individual sialic acid. Here, we developed rapid and specific MRM-MS based platform to determine the content of Neu5Ac and Neu5Gc from human and mammal milk. Initially, total sialic acids were released from glycoconjugates by chemical hydrolysis and enriched using PGC-SPE. Subsequently, Neu5Gc and Neu5Ac were quantified using specific transition such as the ion at *m/z* 87 ($[C_3H_4O_3\text{-}H]^-$) for Neu5Ac and the ion at *m/z* 116 ($[C_4H_7NO_3\text{-}H]^-$) for Neu5Gc in negative ion detection mode. The developed analytical platform was successfully applied to screen of sialic acids in milk. We could compare the absolute contents of Neu5Ac and Neu5Gc of breast milk from four asia countries (Korea, China, Vietnam, and Pakistan) and commercially available milk and infant formulas (3 milk and 10 infant formulas). The level of Neu5Ac in breast milk was greatly higher than that from bovine and goat milk products. Interestingly, Neu5Gc was only found to be high in dairy products about 4.5ng/ml but it was not found in human milk. This platform could be extensively applicable for rapid and accurate quantitation of sialic acid contents including immunogenic NeuGc in the various candidates.

Poster - 118

Development of isotope dilution–liquid chromatography tandem mass spectrometry for the accurate determination of deoxynivalenol and its derivatives in corn flour

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An isotope dilution-liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) method has been stablished as higher-order reference method for accurate determination of major type B trichothecenes, deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), fusarenon-X (FUS-X) and deoxynivalenol-3-glucoside in corn flour. $^{13}C_{15}$-DON, $^{13}C_{15}$-NIV, $^{13}C_{17}$-3ADON, $^{13}C_{15}$-15ADON were used as internal standards and immunoaffinity cleanup was employed to overcome matrix effect. The analytical method was validated by measuring samples fortified with the target mycotoxins at three different spiked concentrations, 100, 400 and 1200 μg/kg. Moreover, the method was also applied to the analysis of DON in corn and wheat in NIST RM 8427 and quality control samples. Repeatability and reproducibility studies showed that the ID-LC/MS/MS method is reliable and reproducible within 6% relative standard deviation. Limit of detections of DON and its derivatives were 0.02-1.80 μg/kg. This method will be applied for the development of certified reference materials.

Poster - 119

Study of the Metabolites and Flavor Characteristics in Different Subtypes of White Tea by Metabolomics Profiling

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This Application Note describes the influence of nonvolatile compounds in white tea on flavor characteristics through an UHPLC-Q-TOF/MS based nontarget metabolomics profiling approach. Profiling of the tea metabolome using UHPLC-Q-TOF/MS followed by feature extraction and alignment resulted in 1,915 metabolite features. Principal component analysis (PCA) and supervised partial least square differential analysis (PLSDA) based on above features demonstrate a clear separation of three subtypes of white tea samples. Up to 99 compounds were identified by matching against authentic standards and databases. Forty-one metabolites exhibit high correlation with flavor; theanine, aspartic acid, asparagine, and AMP are positively correlated with the umami flavor, and flavan-3-ols, theasinensins, procyanidin B3, and theobromine have positive correlations with higher bitterness and astringency flavors. The results demonstrate that metabolomic profiling can be an effective approach to differentiate tea characteristics through characteristic compounds, and that such compounds are potential markers for determining the artificial adulteration and mislabeling of white tea in the market.

Poster - 120

Method Validation for Analysis of Per- and Polyfluoroalkyl Substances in Crab and Fish using LC-MS/MS

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Along with the phase-out of legacy PFASs and their precursors, emerging PFASs such as GenX, F-53B have been used for alternative compounds of PFOA and PFOS. The most commonly used extraction for legacy PFASs is SPE, IPE, SLE and determination methods is LC-MS/MS. In this study, the IPE method which we used for legacy PFASs was applied to perform validation for Simultaneous analysis of 19 legacy and 11 emerging PFASs using LC-MS/MS. Also, this method was applied to crab tissue. Pooled samples were prepared at three concentration levels by adding standard solution. Target compounds were extracted from samples using ion-pairing extraction. Quantitative analysis was performed by LC-MS/MS. The average recovery and precision of legacy PFASs ranged from 78.41% to 122.1% and 2.6% to 13.3%. The average recovery and precision of emerging PFASs ranged from 51% to 129.7% and 4.2% to 28.4%. Through these results, it was confirmed that the reproducibility was well presented in the case of legacy PFASs and the ideal results were found in most PFASs except for some emerging PFASs such as 6:2 FTCA, 8:2 FTCA, 6:2 diPAP. When this method was applied to analyze chinese mitten crab, the mean concentration of emerging PFASs was 2.3 ng/g and the concentration of 6:2 Cl-PFESA in PFAS was 2ppb, which was the highest concentration. In this study, we obtained ideal validation values of PFASs. So the method for legacy PFASs has successfully applied to the emerging PFASs.

Poster - 121

Preliminary study on optimized analysis of I-129 using ICP-MS

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During operation of pressurized water reactors (PWRs), radioactive iodine (radioiodine), one of fission products of uranium, is released into the primary system from defected cladding of uranium fuel and/or tramp uranium deposited on the core surface. Estimation of radioactive inventory of radwaste, required for final disposal, needs determination of I-129 due to its very long half-live (1.7E+07 years). For the same reason, I-129 is used as an indicator for long-term safety of disposal facilities. This paper presents results of preliminary study which was carried out to optimize analytical method using ICP-MS for analysis of I-129 in samples of DAW (Dry Active Waste) generated at domestic PWRs.

Poster - 122

Derivatizaton of pinacolyl alcohol (PA) with various reagents for enhanced analysis by gas chromatography mass spectrometry

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It is very important that the confirmation of chemicals warfare agents (CWAs) and their degradation product. Pinacolyl alcohol (PA) is one of the well-known precursor and degradation product of GD (Soman or 1-methyl-2,2-dimethyl-propyl methylphosphonofluoridation). Underivatized PA is very difficult to identify in the complex sample matrices due to its low boiling point and polarity. Therefore, in this study, we introduced an efficient derivatization methods for the pinacolyl alcohol (PA) using various reagents for the qualitative gas chromatography mass spectrometry analysis

Poster - 123

Optimizing ICP-QMS for determination of uranium isotopic ratio

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The determination of uranium isotopes is important for environmental radioactivity monitoring which investigates the releases of uranium from nuclear facilities. Generally, TIMS and MC-ICP-MS have been widely used for the accurate measurement of isotopic ratio through simultaneous detection and high resolution. Although quadrupole-based ICP-MS (ICP-QMS) has the sequential nature of analysis, but it is much easier to prepare samples, shorter measurement time, high detection limits and more economical than TIMS and MC-ICP-MS. Therefore, ICP-QMS is more appropriate for routine application with large sample numbers.

In this study, experimental and instrumental factors are optimized for precise determination of uranium isotopes using ICP-QMS. The factors of this experiment include detector dead-time correction, dwell time, and sweep numbers used. This results, which optimized factors minimizing the influence of instrumental bias, can be applied to the accurate determination of uranium isotopes in the environment samples.

Poster - 124

Comparison of Matrix Deposition Methods for Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI MSI) of *Drosophila* Brain lipids

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Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) is an analytical technique used to measure the spatial distribution and relative intensity of biomolecules such as lipids, peptides, and proteins in biological tissue samples. For MALDI MSI analysis, it is important to choose the appropriate matrix and how to deposit it to biological tissue. There are two methods of deposing the matrix to the tissue surface: a 'spray method' for spraying a matrix solution into very fine aerosol and a 'sublimation method' for heating a powdery matrix in a vacuum condition. Because the two methods produce matrix crystals of different sizes on the tissue surface, the choice of the deposition method has a significant effect on the results of the analysis.

In this experiment, we deposited 2,5-dihydroxybenzoic acid (DHB) and 1,5-diaminonaphthalene (DAN), the matrices used for the detection of lipids, with the spray and sublimation method. And we compared which method is better for analyzing lipid of *Drosophila* brain.

As a result, the relative abundance of the phospholipids (m/z 720-860) of the overall average spectrum from MALDI MSI was higher than that of 2,5-DHB when 1,5-DAN was deposited by spray and sublimation method. We also obtained a clearer MS image when using 1,5-DAN than 2,5-DHB. In addition, comparing the deposition method using 1,5-DAN, the sublimation method was effective in obtaining a better MS image quality than the spray method. These results suggest that sublimation of 1,5-DAN is the most effective deposition method for lipid analysis of *Drosophila* brain.

Poster - 125

LC-HR-MS/MS Analysis of *E. papyrifera* and its Anti-osteoporosis Activity

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Osteoporosis is a clinical condition characterized by low bone strength that leads to increased risk of fracture. Strategies for the treatment of osteoporosis involve inhibition of bone resorption by osteoclasts and increase of bone formation by osteoblasts. Here, we identified the extract derived from the stem part of Edgeworthia papyrifera that enhanced differentiation of MC3T3-E1 cells to osteoblast-like cells and inhibited osteoclast differentiation of RAW264.7 cells in vitro.

We performed metabolite profiling using by high-resolution mass spectrometry (HR-MS) and tandem mass spectrometry (MS/MS) analysis and identified 10 compounds from the methanolic extract of E. papyrifera stem and duramen. Among them, those already reported by other groups are as follows: daphnodorin B, edgeworoside A, daphnodorin A, and daphnoretin. However, chlorogenic acids, rutin, daphnorin, tiliroside, and rutarensin were identified in E. papyrifera for the first time in our study. The analysis was followed by in-house MS/MS spectral database search to correlate both high-resolution mass spectrum and formula prediction of each component in methanolic extract with those of known compounds in natural-product databases available online.

Taking these observations into account, we suggest that E. papyrifera is an interesting candidate for further exploration as an anti-osteoporotic agent.

Poster - 126

Optimization of Chromatography Conditions for Efficient Isomer Separation and Sensitive Mass Spectrometric Detection of Gangliosides

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Gangliosides are acidic glycosphingolipids which are mainly found in the outer plasma membrane of eukaryotic cells and abundant in central nervous tissues such as brain. Gangliosides are composed of a ceramide (Cer) core and an oligosaccharide head group which contains one or more sialic acid moieties, N-acetylneuraminic acids (Neu5Acs). In analyzing gangliosides by liquid chromatography-mass spectrometry (LC/MS), a proper choice of a chromatographic condition is critical since there are several variations in ganglioside structures based on compositions and structures of oligosaccharide head groups as well as compositions of ceramide cores. Recently, we reported a simple LC method that could effectively separate one of the most abundant ganglioside isomers- GD1a and GD1b- with a conventional C18 column and an ammonium salt additive. In this study, we performed systematic evaluation of various ammonium salt additives in terms of the isomer separation ability as well as the mass spectrometric sensitivity. In addition, roles and effects of ammonium salt additives in LC/MS analysis of gangliosides were suggested based on observations through this evaluation.

Poster - 127

Validation of Analytical Method for Determination of Diacetyl and Acetylpropionyl in e-liquid Using GC-MS

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This study was conducted to develop the analytical method about simultaneous determination for determination of diacetyl and aetylpropionyl e-liquids using GC-MS. The analytical method was validated with respect to parameters such as linearity, limit of detection, limit of quantification, accuracy and precision. The acetone was used as the extraction solution of diacetyl and acetylpropionyl in e-liquid. The limit of detection and limit of quantification results for analysis of diacetyl and acetylpropionyl was 2 ng/mL and 5 ng/mL, respectively. The calibration curve for diacetyl and acetylpropionyl of a range of 5 to 500 ng/mL using a quinoline-d7) as the internal standard (IS) showed a good linearity correlation (>0.999, n=3), respectively. The recovery rate of diacetyl and aetylpropionyl at 3 concentration levels (n = 3) was in range of 87.0 - 108.1%. The accuracy (Recovery %) and precision (% RSD) of analytical method for intra and inter days were 70 to 120% and <20% at quality control (QC) concentrations. The results of recovery, accuracy and precision were studied within the acceptable range according to SANTE (11813/2017) guidelines. Therefore, this method could be efficiently used to the analytical study for determination of diacetyl and acetylpropionyl in e-liquid.

Poster - 128

Urine metabolomics in alopecia areata patient; androgens and bile acids profiling by LC-MS/MS

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An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. Studies on autoimmune disease patients have generally shown an increase in apoptosis, and UDCA, a type of bile acid, is currently used as a potential inhibitor of apoptosis. Previous studies have also shown a decrease in androgen levels in biological specimens of autoimmune disease patients. Therefore, we will develop a simultaneous analysis method of bile acid and androgen that can be associated with alopecia areata, which one of the autoimmune disease, and investigate the level difference through application to sample.

For combined profiling of bile acids and androgens, liquid chromatography-mass spectrometry based analysis method was developed and applied to urine samples of two groups. 40 samples of urine from normal controls and alopecia areata patients were taken to compare the target levels. In this study, liquid–liquid extraction step was performed during the sample preparation. β-glucuronidase/arylsulfatase was also used in the enzyme hydrolysis step. Chemical derivatization was performed for simultaneous determination of androgen and bile acid. All analytes were separated and measured using selected reaction monitoring in the positive ion mode within a run time of 25 min.

In the quantitative profiling, deoxycholic acid and ursodeoxycholic acid level was significantly increased in the alopecia areata patient group. Among androgens, there is no significant difference between two groups.

The mass spectrometry-based quantitative profiling method used herein has great potential for the metabolism study of various inflammatory diseases.

Poster - 129

Universal Sample Processing of Multiple Sample Types for Reproducible Proteomic Sample Preparation

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Cell lysis and protein extraction efficiency constitute the vast majority of variability observed in protein analysis. This, in turn, limits the utility of proteomics in clinical settings. Three of the well described causes of variability are: 1) having different levels of disruptive physical force applied to the sample leading to different level of protein extraction efficiency, 2) being rarely able to reproducibly dissolve proteins with extremely diverse solubility properties, and 3) losing sample and complexity during buffer exchange and sample transfer steps.

In this poster we describe an efficient and universal workflow that resolves these significant workflow variables in processing fresh-frozen and FFPE stabilized tissues by utilizing:

- Covaris Adaptive Focused Acoustics® (AFA®) technology for highly reproducible and efficient sample disruption and extraction
- ProtiFi optimized 5% SDS as a universal protein extraction/solubilization buffer
- ProtiFi S-Trap for rapid protein concentration, contaminant and detergent removal, and in-column protein digestion.

Compared to satandard procedures, the combination of SDS/AFA®/S-Trap significantly increases efficiency, throughput, protein yield, and thus, protein ID rates. In addition, we achieve the goal of reproducible standardized protein recovery in a workflow suited to automated, high-throughput analyses. We anticipate this combined workflow support the translation of proteomics into clinical applications.

Poster - 130

Accurate determination of Mg, Fe, and Si in high purity alumina by inductively coupled plasma optical emission spectroscopy with standard addition method

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Alumina oxide (Al_2O_3) commonly called Alumina has been utilized for diverse industrial applications because of chemical inertness, electrical insulation, and high thermal conductivity, etc. However, impurities in alumina prohibit growth of crystalline or deteriorate the crystal properties. In this study, an analytical method for the accurate analysis of trace elements in high purity alumina (HPA) has been developed. Pressurized microwave-assisted acid digestion method with hydrochloric acid was applied to complete dissolution of alumina powder. After stepwise digestion of 1 g alumina power with 25 % hydrochloric acid at 250 °C for 1 hour, no residuals were observed. For the accurate determination of magnesium (Mg), iron (Fe), and silicon (Si) mass fractions, inductively coupled plasma optical emission spectroscopy (ICP-OES), which was believed to be more robust in high matrix contents, combined with standard addition method (SAM) was used to overcome the matrix effects due to high content of aluminium. For method validation, isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) was applied to the same samples and the results were compared with the developed methods. The measurement results of ICP-OES with SAM were in good agreement with those of ID-ICP-MS except for Si. In addition, NMIJ CRM 8007-a Fine Alumina Powder-high purity were used as a quality control sample and the results were in good agreement with the ceritified values. For the accurate analysis of Si, addtional validation has been carried out and a matrix separation method has been tested. This analytical method can be applied to the assignment of certified values of KRISS alumina CRM and will be submitted as a standard method for the impurity analysis of Mg, Fe, and Si in HPA.

Poster - 131

Feasibility study of uranium isotope ratios for particle analysis by femtosecond LA-MC-ICP-MS

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The isotope ratios of nuclear particles provide the important information related to the nuclear activities. Typically, fission track thermal ionization mass spectrometry (FT-TIMS) and secondary ion mass spectrometry (SIMS) have been used for particle analysis. However, FT-TIMS technique must require the pretreatment of the sample, irradiated by thermal neutrons in a nuclear reactor. Also, SIMS technique has a limitation in analysis of the minor isotopes, such as ^{234}U and ^{236}U, because of isobaric interferences. For these reasons, many laboratories are trying to develop other alternative particle analysis metohds. Our laboratory has been studying the combination of femtosecond Laser Ablation (LA) and Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS) as an alternative technique. In this study, analytical conditions (carrier gas, laser spot size, shot frequency, etc.) were optimized for determining uranium(U) isotope ratios of individual particles by femtosecond LA-MC-ICP-MS. For the improvment of sensitivity, LA system (J200) and desolvation system (Aridus2, cetac technologies) are coupled with the MC–ICP–MS through a connector. The carrier gas (helium) from the LA system and argon gas flow from the Aridus2 are mixed in the connector before entering the ICP. The optimization of the LA-ICP-MS has been performed with NIST 612 glass standard material containing 37.37 ug/g uranium. The integration time of MC-ICP-MS detector was optimized by evaluating the measurement precision of $^{235}U/^{238}U$ ratio. The optimal integration time was 0.524sec with $^{235}U/^{238}U$ ratio measurement precision of 0.21%. Optimized conditions obtained in this study will be applied in real particles of environmental samples in the near future.

Poster - 132

Optimization of chemical separation of nuclear material particle at picogram levels using MC-ICP-MS for nuclear safeguards

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The determination of uranium(U) or plutonium(Pu) isotope ratios in individual particles is important for nuclear safeguards. U and/or Pu isotope signatures are stored in micrometer-sized particles that can be emitted during nuclear processes. In general, fission track-thermal ionization mass spectrometry and secondary ion mass spectrometry have been used for particle analysis. These have some limitations, though. In our laboratory, analytical technique by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS), used for bulk analysis, was studied to isotope ratio analysis of individual particles as an alternative method for particle analysis. Particle samples should be dissolved and have been separated for isotope analysis by MC-ICP-MS. It is highly challenging for the estimation of reliable quantitive analysis and isotope ratios of U with pg levels due to the presence of U backgrounds from experimental environments and procedures. In our chemical separation process of the routine bulk anlysis, the U is around 5 pg. This value is almost same as the analyzed amount of U in spherical U_3O_8 particle sample of diameter 1μm size. In this study, several efforts were performed to reduce the U backgrounds, such as the chemical separation steps were simplified and the frits of UTEVA resin column was cleaned. After then, the U backgrounds were evaluated. The separation process blank was estimated as 0.5 pg levels. In order to decide the optimal elution volume, the elution curve was produced from optimized separation schemes. When the optimal volume of Pu eluent was 1.5 mL and U eluent was 1.5 mL, the recovery yield of Pu, U was 99.8 %, 99.3 %, respectively. In the future, the optimized method in this study will be applied with real U particles in environmental samples for a quantitative analysis and isotope ratio analysis.

Poster - 133

Identification of similarities between antibody drugs and biosimilars using unique peptide

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Antibodies and related proteins now make up the largest and fastest growing category of protein pharmaceuticals. Original antibody drugs and biosimilars are an important class of such proteins.

Biosimilars refer to cloning drugs whose patents have expired. Biopharmaceuticals are not chemically synthesized, but are made protein products from animal cells, yeast, and Escherichia coli. Accordingly, it is not possible to replicate the same product. When making these biopharmaceuticals, it need to be manufactured using living cells, so it cannot make exactly the same product; Only similar products can be made. Therefore, it is essential to confirm the similarity of the biosimilar with the original drug.

Antibody drugs have a common general sequence and unique sequence that shows the characteristics of each antibody drug. The selection of unique peptides based on trypsin cleavage sites will provide a basis for confirming the similarity of antibody drugs.

In this study, we compared the sequences of four antibody drugs, trastuzumab, infliximab, adalimumab, and bevacizumab. We then screened out the unique peptide that only trastuzumab, ifliximab, had. Using Orbitrap Fusion and LTQ-Orbitrap, we want to statistically check the similarity of antibody drugs and biosimilars after analyzing this unique peptide.

Poster - 134

Measurement of mass bias during isotope analysis of uranium particles using secondary ion mass spectrometry

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Environmental Sampling (ES) is employed by International Atomic Energy Agency to monitor undeclared nuclear activities at an aspect of the nuclear safeguards. ES, the analysis technique of swipe samples taken at nuclear facilities, provides the evidence of nuclear activities, such as amounts of nuclear materials and their isotope ratios. ES is classified into the bulk analysis using ICP-MS and the particle analysis using secondary ion mass spectrometry (SIMS) and fission track-thermal ionization mass spectrometry (FT-TIMS). Compared with FT-TIMS, SIMS is a high sample-throughput technique and possible to obtain the distribution of isotope compositions of most recovered nuclear particles. In order to obtain the accurate isotope ratio, the correction for mass bias is an essential step. Mass bias means the deviation between isotope ratios of true value and measured value in the sample. Mass bias occurs due to a variety of reasons such as beam width, size of apertures or slit, secondary ion transmission, detection and so on. Mass bias measured in SIMS is reported to be 0.3 ~ 0.5 % per atomic mass unit (amu) for uranium. In this study, isotope analysis of uranium standard particles by SIMS has been performed to systematically investigate the degrees of mass bias according to uranium enrichment levels and signal intensities measured by electron multiplier, which can be translated to particle sizes. As a result, the degrees of mass bias from high intensity data were slightly larger compared to low intensity data. This indicates that signal intensity, which means particle size, should be considered as a factor for the mass bias corrections for the highly precise and accurate isotope analysis of uranium particles.

Poster - 135

Determination of chlorpyrifos and its products produced by plasma discharge using LC/MS

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Organophosphorus pesticides have been widely used in foods, but they cause problems such as toxicity, accumulation in the body, and environmental pollution. In this study, the degradation of chlorpyrifos, an organophosphorus pesticide, was investigated using ozone generated by plasma discharge instrument. The chlorpyrifos dissolved in water was treated by the plasma discharge instrument for 1hr. Based on liquid chromatography mass spectrometry (LC-MS) analysis, the chlorpyrifos and its products were identified. The chlorpyrifos was completely degraded within 10 minutes. In addition, three by-products, chlorpyrifos oxon, 3,5,6-trichloro-2-pyridinol and diethyl phosphate were newly produced during the plasma discharge process. In order to quantify the amount of chlorpyrifos and by-products produced during the process, a multiple reaction monitoring (MRM) technique was used. Results showed the residues of only 15.9% of chlorpyrifos and its products were remained after 1 hour plasma discharge process. From the quantitative analysis, a degradation pathway of chlorpyrifos was proposed: chlorpyrifos was preferentially converted into chlorpyrifos oxon by oxidation and followed by the formation of 3,5,6-trichloro-2-pyridinol and diethyl phosphate.

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Synergistic effect of heterostructure of Au nanoislands on TiO_2 nanowires for efficient ionization in laser desorption/ionization (LDI) mass spectrometry

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In this work, combination of matrix is presented which consists of semiconductor nanostructure of TiO_2 nanowire and Au nanoparticles. The synergistic effect of the heterostructure consisting of TiO_2 nanowire and Au nanoparticles is analyzed for the ionization of analytes for LDI-mass spectrometry. The heterostructure was made by (1) TiO_2 nanowire synthesis through modified wet-corrosion method and (2) Au nanoisland formation through thermal annealing of sputtered Au layer on TiO_2 nanowire. In order to explain the synergistic effect of the heterostructure consisting of TiO_2 nanowire and Au nanoparticles for the ionization of analytes, thermal properties of the heterostructure was analyzed by using differential scanning calorimetry (DSC). Finally, four kinds of immunosuppressors were analyzed to demonstrate the ionization performance of the heterostructure for LDI-mass spectrometry.

Keywords: Au nanoisland, TiO_2 nanowires, LDI mass spectrometry, DSC, photocatalysis

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Gold-coated magnetic beads for analyte concentration and ionization for LDI-ToF mass spectrometry

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Magnetic particles coated with Au nanoparticles (Au-MAGs) were developed and used (1) for sample concentration and (2) as a solid matrix for laser adsorption/desorption (LDI)-mass spectrometry. The Au-MAGs were prepared by (1) the coating of polystyrene on iron oxide nanoparticles (PS-MNP), (2) the coating of poly-L-lysine on the PS-MNPs (PLL-coated PS-MNP), and (3) the coating of negatively charged Au nanoparticles on the PLL-coated PS-MNPs (Au-MAG). The Au-MAGs was used to concentrate the target analyte by using electrostatic interactions between positively charged GHP9 and negatively charged Au-MAG and the selective interactions such as gold–sulfur interactions between glutation (GSH) and Au-MAG. And then, the concentrated analyte could be ionized for LDI-mass spectrometry. The Au-MAGs was demonstrated (1) to concentrate the target analyte in a sample solution was tested by using electrostatic interactions and the selective interactions between gold and sulfur and (2) to ionize the concentrated analyte for LDI-mass spectrometry.

Keywords: LDI mass spectrometry, iron oxide, Au nanoparticle, solid matrix, dicarboxylic acids, neurotransmitters

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Peptide Sequencing using LDI-TOF MS by Wet-Corrosion Processed TiO_2 Nanowires

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Peptides are small proteins, usually comprising fewer than 40 amino acids connected by amide bonds. The sequencing of peptide refers to the identification of the amino acid sequence from the N- to C-terminals. In matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for peptide sequencing, the collision-induced dissociation (CID) process is a general approach, which uses accerlerated gas molecules for analyte decomposition by introducing collision with deteched peptide ions during the flight to the detector. However, peptide sequencing through MALDI-TOF MS with CID process requires an expensive tandem mass spectroscopy instrument.

Recently, TiO_2 nanowires for LDI-TOF mass spectrometry by the wet corrosion of a Ti plate was reported, which could be used effectively for the quantitative assay of small molecules such as amino acids. In this work, TiO_2 nanowire target plate was fabricated by the wet corrosion process for peptide sequencing by performing both photocatalytic reaction and solid matrix functions. For the photocatalytic decomposition of peptides, the peptide sample was dropped on a target plate containing synthesized TiO_2 nanowire zones and UV was irradiated. Subsequently, the dried target plate was analyzed by LDI-TOF mass spectrometry using the synthesized TiO_2 nanowires as a solid matrix. The feasibility of peptide sequencing based on the photocatalytic reaction with the synthesized TiO_2 nanowires was demonstrated using three types of peptides. In advance, three peptides with different amino acid sequences but the same molecular weight were distinguished through synthezised TiO_2 nanowire target plate by resultant fragment spectrum.

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Investigation of the relationships between experimental parmeters and ionization patterns in paper spray ionization (PSI MS)

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Paper spray ionization(PSI) is an ambient extractive ionization method for mass spectrometry(MS). PSI utilizes a planar triangular-shaped paper as a sampling base as well as an electrospray tip. By using PSI MS, analytes can be ionized in a similar way to electrospray ionization (ESI). However, fundamental aspects of PSI are not as clear as ESI and still need more investigation. In this study, we investigated the relationships between experimental conditions and ionization patterns of PSI by analyzing various analyte molecules including various proteins and oxidizable molecules. Experimental parameters considered include coating materials for paper tip modification such as graphene oxide and metal nanoparticles, spraying solvent compositions, solvent application methods such as dumping and wicking modes. In addition, humidity, oxygen level, and temperature were monitored with the in-house built ion source chamber and these parameters were also considered to understand PSI phenomena.

Keyword: Paper spray ionization / Proteins / Optimization

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Development of chemical fingerprint analysis methods for refill solutions of e-cigarettes by using direct sampling ionization mass spectrometry.

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E-cigarettes have been growing rapidly in popularity and are considered to be less harful than regular tobaccos. However, their health risks are still uncertain. Main chemical ingredients of e-cigarettes are glycerin, propylene glycol, nicotine, food-grade flavoring, benzoic acid, and other contamminants. Chemicals in the refill solutions (e-liquids) and the generated aerosols of e-cigarettes have been analyzed by gas or liquid chromatography mass spectrometry (GC/MS or LC/MS). In this study, we applied direct sampling ionization MS methods to the chemical fingerprint analyses of various e-liquids for e-cigarettes. Direct sampling ionization methods we studied were matrix-assisted laser desorption/ionization (MALDI) and paper spray ionization (PSI). Through this investigation, we optimized a PSI spraying solvent and a MALDI matrix for obtaining proper chemical snapshots of e-liquids. In addition, we also evaluated whether our analysis platforms can be used for the differentiation of e-liquid products.

Keyword: E-cigarettes / Paper spray ionization / MALDI

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Simultaneous quantitative analysis of marker components of in herbal extract using high performance liquid chromatography-tandem mass spectrometry

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Chemical standardization of herbal extract is needed for quality control and to facilitate the design of clinical trials. To address this issue, methods based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) were developed for detection, characterization and quantitative analysis of marker compounds in four herbal extracts and their mixture.

The LC-MS/MS system was operated using an electrospray ionization probe in positive/negative ion mode; multiple reaction ion monitoring (MRM) mode. Eight Marker Compounds were identified in extract by comparing their retention times and the three independent MRM precursor/product ion transitions with those of corresponding reference standards. The ion of monitor ; *m/z* 249.2→231.1 for atractylenolide III, *m/z*=353.2→191.2 for chlorogenic acid, *m/z* 255.1→119.0 for liquiritigenin, *m/z* 417.2→255.2 for liquiritin, *m/z* 579.3→272.2 for narirutin, *m/z*=609.3→301.2 for hesperidin, *m/z* 845.5→799.4 for ginsenoside Rg1, *m/z*=955.5→793.3 for ginsenoside Ro, respectively.

Various validation parameters, such as linearity, limit of detection, limit of quantification, accuracy and precision(intra-and interday variation) were successfully obtained. The linear range of compounds was 0.02-2.0 ug/ml(r^2=0.9998 or higher) and the RSD of the overall inter and inter-day precisions were less than 8%.

This assay showed excellent sensitivity, accuracy and precision and may be used to address the need for quality control and standardization of herbal extract.

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Verification of size determination method using Au standard nanoparticles in single particle ICP-MS.

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A verification of new size determination method using known Au nanoparticles in the size range of 15 nm – 100 nm was studied in single particle inductively coupled plasma-mass spectrometer (sp-ICP-MS). For higher sensitivity and reliability, the level of background subtraction was carefully determined after fitting to a normal distribution, followed by building a correlation curve of average intensity area per particle vs particle radius. At this moment the number of datapoint for each particle was estimated from gaussian profile, assuming normal distribution of ions in plasma. When the results were fitted to an allometric function of $Y=aX^3$ at the optimized level of mean plus $\sigma \sim 3\sigma$, excellent agreement was found, showing a correlation coefficient (R^2) of 0.995<. Furthermore, a window selection method for identifying nanoparticles with multi datapoint profile was tested for mixture analysis, in which the size distribution can be obtained if the window range was selected from the intensity-frequency plot. Conclusively, the formulated method using average intensity area per particle gave a way to determine unknown sizes from the correlation curve using standard Au nanoparticles, instead of aqueous standard solutions. Furthermore, the window selection method showed a potential to extract the size information of nanoparticles with multi datapoints from unknown mixtures.

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Structural elucidation of isomer-specific gangliosides by C18 LC-MS/MS

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Gangliosides, anionic glycosphingolipids containing one to several sialic acid residues, are the most abundant sialoglycans of brain tissue. Gangliosides play an important role in biological functions such as synaptic remodeling, learning formation. Therefore, identification of isomer series (-0, -a, -b, -c) structure is required to understand biological pathway of ganglioside biosynthesis and related molecular mechanism. Here, we elucidated the structure of isomer-specific moleucles obtained from porcine brain gangliosides standard by LC-MS/MS in both negative ion detection mode and positive ion detection mode. Interestingly, negative ion mode MS/MS provides sensitive detection acidic gangliosides with lower background noise whereas positive mode MS/MS produces more detailed fragment ions such as ceramide within the fatty acyl chain and sphingoid base. For example, GM1a isomer structure could be determined by the presence of the diagnostic ion at *m/z* 616.2 $[M+H]^+$ from positive mode MS/MS. In addition, the m/z 290.1$[M-H]^-$ of diagnostic fragment ion from negative mode MS/MS proves the presence of sialic acid. Furthermore, constructed approach was applied to mouse brain tissue, thereby identifying 66 ganglioside compositions including GT1(36:1), GD1(36:1), GT1(38:1) and GQ1(36:1). In addition, the structures of the gangliosides including modifications such as O-acetylation have been elucidated.

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Molecular Structure Characterization of Petroleum Heavy Oil After Cracking Processes Using FT-ICR MS

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Ultra-high resolution FT-ICR MS (Fourier Transform Ion Cyclotron Resonance Mass Spectrometry) is used very often for molecular composition and structural distribution of petroleum heavy oils. In this study, molecular structure distribution of petroleum heavy oil before and after cracking processes using FT-ICR MS. Cracked oils form the two kinds of cracking processes was tested. After the processes, DBE (double bond equivalent) distribution was increased and carbon number distributions are decreased owing to cracking of long side alkyl chains. Some residual oil samples blended with the cracked oils were also tested. From this study, it was founded that any unknown fuel oil sample can be analyzed if it was blended with cracked oils.