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100526 Analysis of thermal products of chlorpyrifos using LC/FTMS（Yoshinari Yamoto）

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100687 DIFFERENTIATION OF FARMED FISH AND WILD SEAFISH USING ISOTOPIC SIGNATURES（Lian Jie Bay）

100781 Level of Dechlorane Plus in ambient air and development of monitoring method（Koichiro MATSUMOTO）

100787 Determination of hydroxylated polycyclic aromatic hydrocarbons in mariner’s urine by high performance liquid chromatography-tandem mass spectrometry（Tetsuya Hirai）

100862 The analysis of olefine and aromatic hydrocarbon in hydrocarbon mixture using Multi-Dimensional GC/GCMS system（LiBin Liu）

100888 ANALYSIS OF 8 KINDS OF ESTROGENS IN ENVIRONMENTAL WATER BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPH HYBRID TRIPLE QUADRUPOLE MASS SPECTROMRTER（hongyuan Hao）

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100471 High-Resolution Tandem Mass Spectrometry Analysis of the Interactions of Oligonucleotides with Selected River Basin Specific Pollutants（Janna Anichina）

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100828 Simultaneous determination of cationic and anionic compounds using a high-speed polarity switching ESI and an online-SPE LC-MS/MS（Shoji F. Nakayama）

No.100036

# SBSE probe desorption GC-IT-MS analysis of degradation products of esfenvalerate obtained by chemical oxidation process

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Keywords: SBSE-GC-IT-MS; esfenvalerate; chemical oxidative degradation

A growing number of investigations have been reported recently showing the widespread occurrence of agrotoxics in the environment,notably in the aquatic compartment. The treatment of these pollutants by oxidative process using hydroxyl radicals (•OH) have been highlighted because of its high efficiency in the degradation of numerous organic compounds and low operating cost. To evaluate the efficiency of this process and monitoring of intermediates and final products, hyphenated chromatographic techniques are indispensable. However, due to the low concentration of pollutants and their degradation products in aqueous medium associated to the incompatibility of the aqueous matrix with conventional GC-MS techniques,some steps of preparation and pre-concentration of the samples are necessary. The SBSE (stir bar sorption extraction) technique combined with hyphenated chromatographic techniques such as GC-MS resulted in rapid analysis, low solvent consumption, higher analytical precision and sensitivity, and has been successfully employed in the extraction of agrotoxics in water at the concentration range of sub-ng $L^{-1}$. However, SBSE-GC-MS analysis requires thermic desorption of the analytes, and nowadays only one commercial SBSE thermal desorption system is available. On the other hand, an approach called "SBSE probe desorption" is being investigated by us as an alternative to integrate the advantages of SBSE and the power of GC-IT-MS (gas chromatography-ion trap-mass spectrometry) as the detection technique. In this procedure, the SBSE bar, containing the analytes sorbed (extracted) is placed into an appropriated probe and thermally desorbed,without using the commercial SBSE thermal desorption system. In this work the degradation of esfenvalerate, a pyretrhoid insecticide, was studied by using a chemical oxidation process and the degradation products were monitored by using SBSE probe desorption GC-IT-MS. Degradation was performed by using 50% hydromethanolic solution containing 45 mg $L^{-1}$ esfenvalerate, pH 11.25, 25 mg $L^{-1}$ of hydrogen peroxide, within a 4 hour reaction period. After degradation process, the products were extracted by SBSE by using stir bars (10mm × 0.5mm, 24 μL PDMS coating, Twister, Gerstel) at room temperature (25$^{o}$C), for 120 min, with 15% methanol, 12% NaCl and stirring at 1000 rpm. After extraction, the stir bar was placed into the probe of a GC CP 3800 Varian, coupled to an ion trap MS Saturn 2000 (Varian). Thermal desorption of the analytes were done at 250$^{o}$C, and the probe heating was held for 18 min. GC analysis were performed on a DB-5ms fused silica capillary column (30 m x 0.25 mm i.d., 0.5 μm film thickness, Agilent). The oven temperature was programmed from 70$^{o}$C (held for 0.5 min) to 300$^{o}$C/min (held for 6 min), at 20$^{o}$ C/min.Helium was used as carrier gas at flow rate of 1.2 mL $min^{-1}$. The MS analyses were done in the scan mode (*m/z* 40 to 450) using electron impact ionization (70 eV). The temperature of transfer line, ion trap and manifold were set at 300$^{o}$ C, 220$^{o}$C and 40$^{o}$C, respectively. By using the SBSE probe desorption-GC-IT-MS method, it was possible to fully identify two products of chemical oxidation of esfenvalerate, 3-phenobenzoic acid and 3-phenoxbenzaldeyde. These compounds were previously described as being metabolites of esfenvalerate, with small estrogenic (endocrine-disrupting) activity and possibly with small environmental impact (McCarthy *et al*., **J. Environ. Monit**. 2006, **8**,197). The structural elucidation of the other oxidation products of esfenvalerate is still in progress.

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Novel aspects:
Study of degradation of esfenvalerate by using a chemical oxidative process. Utilization of SBSE-GC-IT-MS for extraction of degradation products of esfenvalerate and their identification.

No.100037

# Adosorption property of PCB 209 congeners by gamma-cyclodextrin polymer

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Keywords: Atropisomer,Congener specific analysis,GC-MS/MS,PCBs and other toxic substances,Persistent Organic Pollutants(POPs)

Polychlorinated biphenyls (PCBs) production in Japan was started by Kaneka Chemical in 1954. The infamous incident of Yusho, rice-oil poisoning, took place in western Japan in 1968. In 1970's, regulations over PCBs production and use became enforced and PCBs disposal measures have been in operation since 1980's. PCBs waste destruction is still going on today. PCBs were widely used as insulating fluids in capacitors and transformers. Although their manufacture and commercial use have been prohibited in many countries since the 1970s because of their strong toxicity, environmental persistence, and bioaccumulation, large amounts of insulating oils contaminated with PCBs are still being used or are kept without being appropriately treated in many countries, including Japan. In the Stockholm Convention on Persistent Organic Pollutants (POPs), more than 150 countries have agreed to destroy PCBs until 2025. Thus, the efficient and safe treatment of PCB-contaminated insulating oils is a crucial problem from a global viewpoint.
Cyclodextrins (CDs) are a class of cyclic oligosaccharides consisting of several *gamma*-(1,4)-linked D-glucopyranose units. They have a hydrophobic cavity into which a guest molecule of an appropriate size and shape can be incorporated. The ability of CDs to form inclusion complexes with organic molecules has found applications in many areas, including the food and pharmaceutical industries and analytical chemistry. However, in most cases, inclusion complex formation with CDs has been achieved in aqueous media. On the other hand, much less attention has been paid to inclusion complex formation with CDs in nonpolar organic media, because it has been believed that inclusion complex formation in nonpolar organic media would be very difficult due to the unfavorable competition with enormous amount of nonpolar organic solvents against the guest molecules for inclusion into the CD cavity.
We were reported here the removal of PCBs from insulating oil or nonpolar organic solvents by *gamma*-CD polymers as a new adsorbent. Nonpolar organic solvents containing PCBs and other toxic substances were analyzed adsorption charactor which passed gamma-CD polymers solid phase using the GC-MS/MS. PCB 209 congeners were analyzed by product ion obtained by destroying precursor ion using MS/MS method. Other toxic substances were analyzed in the same way. The *gamma*-CD polymers were prepared by the reaction of *gamma*-CD with various kinds of crosslinkers. Among the *gamma*-CD polymers thus obtained, the polymer crosslinked with terephthaloyl units showed the highest adsorption capability towards PCBs. Using this type of polymer (more than 45 wt% of insulating oil or nonpolar organic solvents) as an adsorbent, PCB 209 congeners and other toxic substance, whose initial concentrations were 1〜100 ppm, were completely removed from isooctane solution.

Novel aspects:
Nonpolar organic solvents containing PCBs and other toxic substances which passed gamma-CD polymers solid phase were analyzed congener-specifically using the GC-MS/MS.

No.100078

# Charge exchange ionization in reversed-phase liquid chromatography-atmospheric pressure photoionization mass spectrometry of hormones

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Keywords: APPI, charge exchange,

Atmospheric pressure photoionization (APPI) has been introduced as an innovative ionization source for LC-API-MS systems to broaden the range of analytes that could be determined using LC-MS even if having weak polarity. From the literature data it is obvious that APPI provides a better ionization for the non-polar analytes than ESI and APCI. Furthermore, photoionization is less susceptible to ion suppression compared to ESI or APCI. Several methods employing APPI-MS detection have been suggested for the analysis of environmentally relevant estrogens, predominantly with toluene as dopant.
Under reversed-phase liquid chromatography conditions, toluene has become a dominant solvent used in the dopant-assisted photoionization (DA-APPI). Toluene allows charge exchange in solvents with low proton affinities, however, in solvents commonly used in reverse phase chromatography (acetonitrile, methanol); ionization via proton transfer prefers to via charge exchange reaction. The use of APPI-MS with a dopant enabling ionization via charge exchange will be evaluated for the determination of seven environmentally relevant estrogens and progestogens (preferably hormones cited in U.S. EPA contaminant candidate list 3) in drinking and river water. Finally, the comparison between APPI-MS using ionization via proton transfer (toluene as dopant) or charge exchange (e.g. anisole, chlorbenzene, brombenzene, 2,4-difluoroanisole, 3-(trifluoromethyl)anisole) will be performed in terms of sensitivity and ion suppression assessment.
The financial support from the Grant Agency of the Czech Republic (Grant Reg. No. GAR P503/10/0975) is gratefully acknowledged.

Novel aspects:
The use of charge exchange reactions in APPI-MS/MS detection of hormones under reverse-phase HPLC conditions.

No.100110

# Identification of Biodegradation Products of High-Molecular-Weight Perfluorinated Compounds Using Two-Dimensional Liquid Chromatography/High-Resolution Mass Spectrometry

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Keywords: 2D-LC, Orbitrap, PFCs, biodegradation

Perfluorinated compounds (PFCs) have been considered an environmental problem. Early studies on PFCs focused on the presence of perfluoroalkyl carboxylic acids (PFCAs) and sulfonic acids (PFSAs) in environmental matrices and their possible biological effects. However, more complicated PFCs were usually used rather than simple molecules such as PFCAs and PFSAs. There is limited information about such complicated PFCs. Although major fluorochemical companies now have phased out of production and use of PFOA, PFOS and PFOS-related products, PFOA and PFOS still have been found in various contexts. Both fluorochemical products and environmental samples always contain complicated matrices. It is difficult to determine what kinds of PFCs really exist.

In the past few years, two or multidimensional liquid chromatography has been attractive for analyses of complex mixtures. Multidimensional chromatography coupled with mass spectrometry notably offers comprehensive analysis and has been applied to the characterization of natural products and industrial materials. In this study, we used an off-line 2D liquid chromatograph coupled with a high-resolution mass spectrometer (2D-LC/HR-MS) to identify commercial fluoroproducts and their biodegradation products.

Fluoroproducts examined consisted of four products. Three of them were products sold before the regulation. All of them were purchased in Japan. Liquid samples were diluted in water by 3000-fold. Activated sludge was added to the diluted samples at a concentration of 30ppm. The samples were transferred in closed containers, which were made to be able to supply oxygen for aerobic biodegradation. Ten-mililiter portions of samples were taken from each stirred sample once a week for a month. The portions passed through solid phase extraction cartridges, OASIS WAX (Waters), to extract PFCs. PFCs were eluted from the cartridges with 0.1% $NH_4OH$ methanol. The eluates were analyzed by liquid chromatograph/mass spectrometer, Ultimate 3000/Exactive (ThermoFisher).Ultimate 3000 and Exactive were used for acquisition of mass spectra with a high resolving power of 100,000. In addition, Acquity and Xevo TQ (Waters) were used for quantitation of PFCAs and PFSAs and acquisition of product ion spectra. Two-dimensional LC was carried out with two different kinds of columns. A TSK-Gel ODS-100S from TOSOH and an Epic-FO column from ES-industries were used for chromatography.

Acquired mass spectra with single LC included numerous ions. The interpretation of massspectra was, therefore, very difficult. Two-dimensional LC could effectively separate these compounds depending on the interaction strength with two different types of columns. The mass spectra that generated from separated compounds were easier to interpret than the spectra from unseparated compounds. Fragment ion spectra were also acquired.

The chemical structures were determined from the interpretation of the data of samples before biodegradation. A dominant ion with an *m/z* value of 1574.2809 was found in one product examined. Because three fragment ions that had equal spaces of 357.007 were found in the observation with collision gas, the compound was the molecule that had three $C_4F_9SO_2NCH_3C_2H_4O$ groups. If triisocyanate such as Tolonate reacts with an alcohol, $C_4F_9SO_2NCH_3C_2H_4OH$, the chemical formula of the molecule yielded will be $C_{45}H_{60}N_9O_{15}S_3F_{27}$. The exact mass of the ion is 1574.2867. The two values were well-matched.

Biodegradation products were also examined by analyses of the samples after biodegradation. Two out of four products examined showed unequivocal increase of concentration of PFCAs. Although the above-referenced sample did not show any increase of PFCAs and PFSAs, a degradation product that had a carboxyl group at the terminal was identified. The result complemented the presumption of original structure. However, further degradation structures such as sulfonamide were not found.

Two-dimensional LC/HR-MS demonstrated the occurrence of biodegradation of huge PFCs to small PFCs in aerobic biodegradation. We need further investigation of fluoroproducts that have been used so far.

Novel aspects:
Two-dimensional liquid chromatography and high-resolution mass spectrometry could demonstrate the occurrence of

biodegradation of huge PFCs to small PFCs in aerobic biodegradation.

No.100145

# Yellow Sand and Seawater Interaction from viewpoint of Silica Speciation

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Keywords: Yellow sand, FAB-MS, silica speciation

1. Yellow sand is a kind of loess and distributed in N. W. China. It is carried eastward by prevailing winds and passes over China, Korea, and Japan. Sulfur, carbon monoxide, and other toxic pollutants including heavy metals (such as mercury, cadmium, chromium, arsenic) often accompany the dust storm, as well as viruses, bacteria, and combustion products. The dust is known to cause a variety of health problems. In Japan, large amounts of yellow sand is mainly transferred in spring. If yellow sand does not contain toxins, it could be a good supplier of silicic acid (silica) to the Sea of Japan, especially in spring. Silica species are nutrients for diatoms in seawater. During spring, silica tends to be lacking due to "blooming" in some regions. The authors have studied dissolution states in aquatic phases, particularly seawater. In this study, the behavior of silica species in yellow sand with model solution of seawater (sodium chloride (NaCl) solution) was examined under several conditions, and the role of yellow sand is considered.
2. Sample of yellow sand was obtained at Hotan in N. W. China by 200 mesh sieve. A portion of yellow sand (0.2g) was shaken with 20 mL of several kinds of sodium chloride at 25$^{o}$C for several days. The silica concentration was determined using molybdic yellow methods of spectrophotometrically. The chemical species of silica was identified by FAB-MS spectrometry (JEOL JMS 700) (negative ion detection mode). The conditions for mass spectrometry were as follows: for FAB(Xe, 1 mA emission), the resolving power was m/m=1000 and mass range was 0-1000.
3. **pH dependence** The pH of solution was changed from 0.33 to 14.1 and shaken for 7 days. A change in pH from 0.52 to 14.2 of solution containing yellow sand with a 0.5 mol $L^{-1}$ sodium chloride solution was attained. However, the pH before shaking was between 3.2 and 9.0 and remained within 8.1-8.2 after shaking for 7 days. The silica concentration in NaCl was almost a constant 30 $\mu$mol $L^{-1}$. When the pH of the solution was 0.5, 12.4, and 14.2, silica concentration was 1320, 158, and 81 $\mu$mol $L^{-1}$, respectively. When the yellow sand was poured into a NaCl solution, pH equilibrium was attained and NaCl solutions with various pH, were almost constant. In this pH range, the silica species in solution were observed as [monomer-$Na^{+}$ complex]$^{-}$, [monomer-$Ca^{+}$ complex]$^{-}$, dimer, cyclic tetramer and linear tetramer. When pH of the solution was 0.5, peak intensity of silica species was extremely low and the same species were observed. When pH solutions were 12.4, and 14.2, the silica species were clearly observed. The silica concentration in solution with pH 0.5 was high, but silica species not clearly observed. It is considered that silica could be dissolved as particles in the low pH solution but it could be dissolved as ions in high pH.
**Time dependence** When yellow sand was dissolved for 0, 1, 3 7, and 14 days, the silica concentration increased. For 1 day, dimer and linear tetramer were observed compared to cyclic tetramer. But for 3 days and more, dimer and cyclic tetramer increased compared to linear tetramer. When the solution did not attain equilibrium for 1 day, yellow sand dissolved as linear tetramer first, then changed to cyclic tetramer. According to our previous studies, it has become clear that "food" of diatoms was dimer and linear tetramer. When yellow sand falls into seawater, the dimer and linear tetramer would be produced immediately, at the surface of seawater.

Novel aspects:
From our measurement by FAB-MS, it becomes clear that the dimer and linear tetramer are produced at the surface of seawater, when yellow sand falls into seawater, .

No.100152

# Current status of organophosphorus compounds contaminants in Maizuru Bay, Japan

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Keywords: organophosphorus compounds, water, sediment, mussel

Organophosphorus (OP) compounds which have been utilized as flame retardants,plasticizers, stabilizers, antifoaming and wetting agents, and additives inlubricants and hydraulic fluids etc. are the useful chemical compounds and are used in the various areas. It is well known that aquatic environment has contaminated by volatilization, leaching or abrasion from the broad applicationrange of these compounds. In this study, the concentrations of eight species oforganophosphate esters (OPE) and OP pesticides (diazinon, fenitrothion,chlorpyriphos and iprobenphos) are measured in water, sediment and green mussels from Maizuru Bay and the distribution of these compounds in waterenvironment is discussed.

Water sediment and mussel samples in Maizuru Bay were taken from July 7th to 15th, 2009. Subsurface water samples were taken in 7 sites and thesurface sediment samples (0 5 cm) were taken using a Ekman-Birge type bottom sampler in 13 sites. The mussel (*Mytiusgalloprovincialis*) samples were taken at 9 sites. The shell lengths of the green mussels were in the range of 380 - 750 mm. Three mussel samples in eachsite were homogenated before analysis. Water samples were stored in a fridge at3 $^{o}$C and their samples were analyzed within a few day. Sediment andmussel samples were stored in a freezer at -20 $^{o}$C for until chemicalanalysis.

Water samples were extracted with dichloromethane by shaking for10 min. The aqueous layer was dried with anhydrous $Na_2SO_4$and was concentrated up to 0.5 ml after the addition of Atradine-$d_{25}$ as an internal standard. The analytes were measured by GC-MS. OPs in sedimentand mussel samples were extracted with acetone. After centrifugation, thesupernatants were cleaned by addition of distilled water, celite and zincacetate. After removal of suspended matters by filtration, the analytes inliquid layer were extracted dichloromethane. After the addition of Atradine-$d_{25}$,the organic layer was concentrated up to 0.5mL. The analytes were measured by GC/MS.

The distribution of OP compounds in Maizuru Bay was surveyed. The concentrations of OP in water samples from Maizuru Bay were in the range of 2.7 62 ng/L, which these level was under a thousandth of acute toxic level for aquaticorganisms. The concentrations of OPEs were high the order of TBXP > TDCPP >TCEP > TBP > TCP > TPP > TEP. This trend is similar to those in 1976-1996. Diazinon, fenitrothion and chlorpyriphos of OP pesticides were detected in water samples.

The concentrations of OP compounds in sediment from Maizuru Bay were in the range of <0.5 - 56 ug $kg^{-1}$ dry weight (dw). The patterns of OPEs in sediment are classified two groups. The patterns of OPEs concentrations in a group arecharacterized that OPEs concentrations are high the order of TBXP > TDCPP orTBP, and the other OPEs are similar concentrations. The patterns of OPEs concentrations in the other group characterized that TDCPP concentrations isthe highest among OPEs. Diazinon was detected in all sediment samples from Maizuru Bay and the concentrations of diazinon were in the range of 1.8 and 71 ug $kg^{-1}$ dw. The detection frequencies of fenitrothion andchlorpyriphos were low in sediment.

The concentrations of OP in mussel fromMaizuru Bay were in the range of <0.5 and 34 ug $kg^{-1}$ wet weight.The concentrations of OPEs were high the order of TBP > TDCPP >TCP >TBXP = TPP = TCEP.

The partition rate between water and sediment (Kws) of diazinon and fenitrothion were in the range of 200 and 1300, and in the range of 200 and 300, respectively and the partition rate between water and biologicalsamples (Kwb) of diazinon and fenitrothion were in the range of 700 and 3300, and in the range of 450 and 700, respectively, suggesting that these pesticides prefer biota to sediment.

Novel aspects:
Organophosphate esters and pesticides were detected in water, sediment and mussel samples from Maizuru Bay, Japan.

No.100186

# Tandem SPE clean up/extraction: strategy to minimize matrix effects on LC-MS determination of endocrine disrupters and pharmaceuticals in sewage samples

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Keywords: Emerging Contaminants; Matrix Effects; Sewage

In recent years, a variety of organic compounds, also called emerging contaminants, have being detected by LC-MS, at low concentrations (ng L-1), in samples of surface water, wastewater, groundwater, and even drinking water [1-3]. According to Taylor [4], the matrix effect is the "Achilles' heel" of the HPLC-MS techniques. Therefore, it is extremely critical to evaluate and/or minimize the influence of complex matrix composition on the HPLC-MS responses of analytes. Few articles have evaluated thoroughly the matrix effect in analysis of emerging contaminants in sewage samples. In this work a new method for the determinationof three endocrine disrupters (estradiol, ethinyl estradiol, and bisphenol A)and five pharmaceuticals (sulfamethoxazole, trimethoprim, diclofenac, bezafibrate and miconazole) in raw and treated sewage samples was developed and validated. The method consisted of the application of tandem SPE procedure that uses both a strong ion exchange sorbent (SAX) and a modified divinylbenzene-pyrrolidone sorbent to reduce the levels of linear alkylbenzenesulphonate (LAS) and to concentrate the analytes of interest from sewage samples, prior to analysis by liquid chromatography combined with high-resolution mass spectrometry (LC-HRMS). The influence of matrix composition on the ionization efficiency, the SPE recoveries, and the sensitivity of the method was determined. The SAX sorbent was capable of significantly removing LAS content in sewage samples extracts. It was also capable of retaining the analytes that were eluted with ethyl acetate with recoveries that varied from 17 to 35% (CV <9%). This approach was very efficient at minimizing the matrix effects. The sum of the recoveries from both sorbents varied from 30 to 70% (CV <9%). The mass spectrometry detection was performed using a LC-ESI-IT-TOF/MS instrument working at high-resolution (10.000 FWHM) and high mass accuracy (< 5 ppm). The instrumental limits of quantitation varied from 0.4 μg L-1 to 3.3 μg L-1. The method limits of quantitation ranged from 3.3 ng L-1 to 41 ng L-1. The method was successfully applied to the determination of analytes in raw sewage samples at the Arrudas Sewage Treatment Plant, Belo Horizonte, Brazil and also to evaluate the efficiency of different experimental sewage treatment systems.

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Novel aspects:
The work uses tandem SPE (SAX+HLB). This approach was efficient at removal of LAS and to minimize their matrix effects on ESI responses of analytes in sewage samples extracts.

No.100215

# Simultaneous analysis of anionic, amphoteric and non-ionic surfactants using ultra-high speed LC-MS/MS

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Keywords: surfactant, LAS, Betain,Heptaethyleneglycoldodecylether,TQ

## Introduction

Surfactant chemistry has made a considerable impact on a number of household products including detergents, shampoos and toothpaste. Products are generally classified by the type of each hydrophilic substructure into anionic, cationic, amphoteric and non-ionic surfactants. Either anionic or non-ionic surfactants are typically used as synthetic detergents, however, to better elucidate the potential risk in environmental samples, mainly in agricultural soils and sediments, methods need to take into account a range of surfactant chemistries. Current surfactant monitoring methodologies tend to focus on a specific surfactant. Here, we have developed the simultaneous analysis method for typical anionic, amphoteric and non-ionic surfactant using LC-MS/MS.

## Method

Commercially available surfactants were used for this experiment. Standards of surfactants were diluted with water to an appropriate concentration and then determined by LC-MS/MS. As an LC-MS/MS system, UHPLC was coupled to triple quadrupole mass spectrometer (Nexera MP with LCMS-8030, Shimadzu Corporation, Kyoto, Japan). Separation was achieved using a YMC-Triart C8 column (100mmL., 2.0mmI.D., 1.9um particles) and column oven temperature was maintained at 40 C. Samples were eluted at flow rate 300uL/min with a binary gradient system; the mobile phase consisted of (A) 10mM ammonium acetate buffer and (B) mixture of 10mM ammonium acetate / acetonitrile / isopropanol (1/4/5). LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode with ultra-fast polarity switching.

## Preliminary data

The following standard surfactants were selected and analyzed; anionic surfactant: linear alkylbenzene sulfonate (LAS) C10-C14 mixture, amphoteric surfactant: EMPIGEN BB Detergent (Betaine) C10, C12, C14 mixture and non-ionic surfactant: heptaethyleneglycoldodecylether (HEDE). Full scan measurement by flow injection analysis (FIA) was conducted to determine the optimum ionization polarity of target compounds followed by MRM transition optimization by FIA. As a consequence, all LASs were detected as the de-protonated ions and m/z 183 was selected as the product ion of MRM transitions for all LASs (C10-C14). All Betaine were detected as protonated ions and m/z 104 was selected as the product ions of MRM transitions for all Betaine (C10, C12 and C14). HEDE yielded the protonated ion as the precursor and m/z 133 was selected as product ion for MRM transition. As compounds selected in this experiment formed either positive or negative ion, high-speed polarity switching is an important parameter to consider in developing an optimized method. The dilution series of these compounds were analyzed and all compounds were detected at sub ppb level with excellent linearity. In addition, the quantitative analysis of real world sample using the kitchen detergents and liquid soap was achieved using this method

Novel aspects:

Simultaneous analysis method of anionic, amphoteric and non-ionic surfactant using ultra-high speed polarity switching technique of LC-MS/MS was developed.

No.100271

# POLLUTION OF MOSCOW AIR: GC/MS STUDY OF SNOW SAMPLES

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Keywords: organic pollutants, GC/MS, ICP/MS, high resolution mass spectrometry, snow

Moscow is the largest European city with population about 15 millions and hundreds of enterprises. More than 4 millions cars are registered in Moscow. However just the most common atmospheric pollutants are monitored in the city at the regular basis. To propose a list of priority pollutants for the atmosphere of Moscow 16 snow samples were collected along the perimeter (109 km) of the Moscow belt road at the end of March 2011 and 2012. Snow is an excellent preserving matrix allowing keeping the majority of chemical compounds including not very stable ones (e.g.phenols). In the countries with cold climate analysis of snow gives a chance to estimate long term atmospheric pollution (several months).
Mass spectrometry was used as an analytical tool to identify individual organic compounds (gas chromatography/mass spectrometry, GC/MS) and the most environmentally relevant chemical elements (inductively coupled plasma with mass spectrometric detection, ICP-MS). Sample preparation was carried out according to US EPA 8270 and 200.8 Methods, while LECO Pegasus IVD and Agilent7500c instruments were used correspondingly. Both target and non-target approaches were used. As a result more than 500 organic compounds belonging to various classes were identified in each sample. Besides classic pollutants like PAH, PCB, phthalates several classes of other anthropogenic contaminants including, organophosphates, esters of N,N-diethylcarbamodithioic acid, various nitrogen and sulphur containing compounds, antioxidants, and some others were represented by a number of compounds. Quite surprising was the detection of several compounds with dichloromethyl group in 2012 samples, including dichloronitromethane as the major ingredient. The confirmation of the identification was obtained by parallel analysis of all the samples with LECO Pegasus GC-HRT instrument with the resolving power exceeding 60000. Several valuable issues concerning reliability and new possibilities for the identification of new compounds were discovered when dealing with a high resolution instrument.
The levels of organic compounds using internal standards as well as the levels of chemical elements were quantified. The data obtained allow estimating atmospheric pollution in Moscow in the period between December and March and proposing a draft list of priority pollutants for the atmosphere of Moscow.

Novel aspects:
A representative list of organic pollutants in the atmosphere of Moscow was created for the first time using high resolution mass spectrometry.

No.100299

# Limitations of a commercially available plasma air purifier

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Keywords: Non-thermal plasma, Cleaning efficiency, Air-cleaning

The molecular processes in a commercially available AC, driven plasma air purifier (PAP) were studied in detail. Such air purifiers are supposed to break down all air contaminants to small, nontoxic molecules (e.g. $H_2O$ and $CO_2$). However, the degradation mechanisms in such PAPs are not known yet. In this study, we examined the exhaust of a commercially available PAP to determine its efficiency and the molecular processes taking place. Three different classes of substance were studied: environmental toxins representative for of low MW molecules, a high-mass protein, and various bacteria to represent very high masses. One goal of this project was to examine the limitations of such commercially available air purifying systems.
A setup was designed in such a way that the PAP could be studied under realistic conditions, simulating common heating and ventilation systems. Phenanthrene and methyltriclosane were chosen as small molecules. Bovine serum albumin (BSA) was chosen as a model high mass protein. *Legionella Pneumophila* and *Bacillus anthracis* were used to cover the class of airborne infectious agents. The sampling method was adapted to the respective compounds. Adsorption tubes were used for the low MW molecules; bubbling 10% of the PAP exhaust through water was used to quantify the amount of degraded BSA; *L. pneumophila* and *B. anthracis* were sampled using agar strips, which were afterwards wrapped in sterile containers and incubated.
The study of environmental toxins using GC-MS showed a degradation of 31.0% and 16.9% for phenanthrene and methyltriclosane, respectively (relative error, 13%). However, no characteristic degradation products could be found. Therefore, a mass balance with methyltriclosane was conducted which yielded surprising results. On the 4 copper electrode surfaces of the PAP exactly 17% were methyltriclosane found. Since the degradation was determined by performing an experiment with and without activated PAP, the decreased amount of methyltriclosane was considered as degraded. However, our experiments do not support the hypothesis of degradation for small molecules anymore.
Measuring the PAP degradation performance of BSA showed a reduction of 81.1–30%, when comparing experiments with and without the plasma activated. The copper electrodes showed a white film after the experiment with the plasma activated. After dissolving the white precipitate in water, LC-MS and MALDI-MS experiments identified it as BSA. 1% of white and water insoluble crystals which were identified as polymerized BSA using MALDI-MS equipped with a high mass detector. The reduction in amount of various bacteria showed, that the PAP is capable of reducing aerosolized bacteria in air. However, it seems that the reduction is mainly occurring due to adherence to the copper electrodes.

Novel aspects:
Efficiency study of a commercially available plasma air purifier over a wide range of different substances

No.100360

# Multi-component quantitative analysis of pharmaceuticals and personal care products in the environment by LC-MS/MS with fast polarity switching

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Keywords: LCMS-8080, higher sensitivity, multi-component quantitative analysis , fast polarity switching, and pharmaceuticals and personal care products (PPCPs)

Pharmaceuticals and personal care products (PPCPs) constitute a group of emerging contaminants which have received considerable attention in recent years. Monitoring of PPCPs in the environment is vital as many of these compounds are ubiquitous, persistent and biologically active with recognised endocrine-disruption functions. Given the hazardous nature of these compounds, there is a need to provide fast and sensitive multi-residue methods that are able to analysis multiple classes of compound within one analytical procedure. Here we report a new multi-residue UHPLC-ESI-QqQ method that utilises fast polarity switching with an optimised chromatographic gradient that removes matrix effects and results in excellent ng/L detection levels. Furthermore, we evaluate the performance of polarity switching in comparison to dedicated single polarity experiments.

Natural river and lake water was collected from the Shiga region (Japan) and spiked, without any sample pretreatment, at a range of concentration levels (1 - 10000 ng/L) with 15 PPCPs (e.g. Carbamazepine, Dehydronifedipine, Gemfibrozil, Triclocarban). Separation was achieved using a Shim-pack XR-ODS III column (50 x 2.0 mm, 1.6 μm) maintained at 40 °C on a UHPLC system, Nexera (Shimadzu, Japan). The method was maintained at a flow rate of 0.4 mL/min with mobile phase A: water/0.1 % formic acid and B: acetonitrile. The gradient (detailed in preliminary data) was optimized to minimise matrix effects. A higher sensitivity triple quadruple mass spectrometer, LCMS-8080 (Shimadzu, Japan) operating in SRM mode with fast polarity switching (20 msec) was used for the detection of positively and negatively charged analytes.

As a result of the complex matrix PPCPs are present; the occurrence of ion suppression/enhancement is commonly described in literature and results in reduced MS/MS detection limits and inferior precision. For this reason, a gradient was optimised that focused target analytes at the head of the chromatographic column while allowing the interfering environmental matrix to be eluted; this resulted in excellent recoveries of around 100 % for almost all studied compounds. This was achieved using a gradient which held the aqueous mobile phase at 100 % for 6 min, and subsequently increased the organic mobile phase to 80 % over 10 minutes and then to 100 % organic mobile phase.

PPCPs encompass a wide range compound classes and chemical properties and consequently it is necessary to employ both positive and negative electrospray ionization for complete analysis in a single run. All compounds were measured by SRM with fast polarity switching (20 msec) for multi-component analysis. Excellent limits of quantification were achieved in the range 1 - 50 ng/L for nearly all studied compounds, with outstanding linearity ($R^2 > 0.999$).

To evaluate the capability of polarity switching the data quality obtained was compared to dedicated negative or positive analysis. Comparisons were made by selecting compounds that prefer positive (Carbamazepine and Dehydronifedipine) and negative ionisation (Gemfibrozil and Triclocarban). Findings showed that the data quality obtained during polarity switching experiments was directly comparable to that achieved during dedicated positive or negative analysis. In addition, long term stability was investigated by making 100 injections over >10 hours, with polarity switching presenting excellent stability.

Novel aspects:
Fast polarity switching results have been equivalent to dedicated single polarity experiments for the analysis of PPCPs in environmental samples.

No.100364

# Screening and library search of Environmental pollutants in Japanese environmental water using LC-MS/MS

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Keywords: PPCPS Environment LC/MS/MS Water

Introductions

In recent years, many kinds of chemicalsubstances are polluting environmental water. When people take a medicine, useinsect repellent, or apply cosmetics, these chemicals flows into the water inthe environment, finally. These compounds are called PPCP (Pharmaceuticals andPersonal Care Products) as a whole. Furthermore, it is known that thepesticides originally used for agricultural products are pollutingenvironmental water. These compounds are also contained in PPCP in a broadsense.

The diversity and the numerousness of thesecompounds make method development challenging. But In order to properly assessthe effects of these compounds on our environment, it is necessary toaccurately monitor their presence. Liquid Chromatography coupled to tandem MassSpectrometry (LC-MS/MS) is able to analyze polar, semi-volatile, and thermallylabile compounds covering a wide molecular weight range, such as pesticides,antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfectionby-products etc. In addition, state-of-the-art LC-MS/MS instruments operated inselective Multiple Reaction Monitoring (MRM) mode, offer unmatched selectivityand sensitivity to quantify PPCP reproducibly at trace levels without timeconsuming and extensive sample preparation.

AB SCIEX QTRAP$^{fi}$4500 LC/MS/MS System can measure many compounds simultaneously because of its fastMRM scanning, and its can measure compounds high-sensitive because its hasQ-jet$^{fi}$2 ion guide.

Morerecently, it is discussed that detection only by MRM cause false positive andfalse negative. To avoid false detection, one useful process is library search fora fragment ion spectrum. This process judges positive or negative by comparingthe spectrum of standard with the spectrum of the compound detected in thesample. At 1ch MRM, while judging from one fragment ion, by the library searchusing a spectrum, a judgment is performed from all fragment ions which thecompound has. Therefore, library search can do more precise judging.

ABSCIEX QTRAP$^{fi}$ 4500 LC/MS/MS System can acquire fragment ion spectrumfast and sensitive because its has Linear Ion Trap technology with Linear Accelerator$^{TM}$ Trap.And AB SCIEX has fragment ion spectrum library that contains large number ofcompounds.

LC-MS/MSis suitable for measurement of PPCP, and judgment by library search isdesirable. Therefore, AB SCIEX QTRAP$^{fi}$ 4500 LC/MS/MS System suitablefor PPCP screening.

Methods andresults

20 Japanese environmental water samples wereinjected directly into AB SCIEX QTRAP$^{fi}$ 4500LC/MS/MS System to detection PPCP at low parts-per-trillion (ppt) levels(ng/L). 4500 quick scanning speeds supported simultaneous analysis of 304compounds (273 for positive, 31 for negative) were acquired by using MRM. EnhancedProduct Ion Scan (EPI) was used for acquiring the fragment ion spectrum at pptlevels, and "Mega-library" spectrum library was used to judge compoundsdetected by MRM were true or false. EPI is high-sensitive and high-speedfragment ion scan mode, supported by Linear Ion Trap technology with Linear Accelerator$^{TM}$ Trap."Mega-library" is fragment ion spectrum library that contains over 1240compound’s spectrums.

From Japanese environmental water, somecompounds were detected by MRM. The detected compounds did not related samplingpoint. According to results of the library search, some compounds were trulydetected, but some compounds were false positive.

As a conclusion, thePPCP exist Japanese wide area environmental water. So it is necessary tomeasure much more samples to know a trend, such as regionality.

Novel aspects:

Fast Aqcuisition time, High Sensitivity, Direct measurement, Over 100 compounds

No.100371

# OPTIMIZATION OF SOLID PHASE EXTRACTION FOR PERFLUORINATED COMPOUNDS ANALYSIS IN WATER SAMPLE

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Keywords: Micropollutant, Perfluorinated compounds (PFCs), PFOA, PFOS, Solid Phase Extraction

In recent years, perfluorinatedcompounds (PFCs) have appeared as a new class of global contaminants. Researchershave reported PFCs contamination in surface water, tap water and bottled water aroundthe world. They are particularlydifficult to deal with once released into the environment, because they do notbreak down easily, they can travel long distances carried by air or in water and they can accumulate in human and animal tissue. To understand the sources and fate of thesecompounds, it is essential to optimize the analytical methods for a wide range of perfluorinated compounds in water sample. Solid phase extraction (SPE) coupled with high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) is generally used to analyze PFCs in environmental matrices. Researchers have reported the difficulties in analysis of short chain (C4 C6) and long chain (C10 C12) PFCs. The ineffective SPE procedure and matrix interferences were the two major difficulties for analyzing environmental water sample. To overcome these problems, the optimizationof SPE process is needed. The objective of this study was toevaluate the optimum SPE condition for two ion exchange (OasisWAXand PresepPFC-II) cartridges for analyzing 12 PFCs in water sample. These cartridges were also compared withPresepC-Agri ($C_{18}$) and OasisHLB, which are generally used for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) analysis. Several options for optimizing SPE were evaluated such as adjusting sample loading flow rate, washing sample's bottle by methanol, optimizing cartridges drying step, adding matrix washing step, conducting sequential elution, adding volume of solvent and optimizing nitrogen purging. Ion-exchange cartridges (OasisWAX and PresepPFC-II) were showed better result comparing to both PresepC-Agri ($C_{18}$) and OasisHLB. Flow rate 10 mL/min showed better recoveries of most chemicals comparing to flow rate 5 and 20 mL/min. Comparison of drying cartridges and without drying cartridges was conducted for both PresepPFC-II and OasisWAX. The average increase of all twelve chemicals was 9%. An experiment was conducted for washing the sample bottles two times after loading to the cartridges. Five milliliter of methanol (2 times) was applied for each washing. Recoveries of C10 C12 increased by 2%, 12% and 33%, respectively for both cartridges. The elution was done three times by using 2mL 0.1%NH4OH in methanol each time. More than 97% of all compounds were eluted in the first elution. An experiment on the effect of PFCs lost during nitrogen purging step was also examined. There was no significant in the loss of PFCs during nitrogen purging for 1 hr, 2 hrs and 3 hrs. The solid phase extraction was optimized for analysis of twelve PFCs in water sample, especially tap water and river water (with low matrix). Coupled with the use of HPLC-MS/MS, a method detection limit in the range of several tens of parts-per-quadrillion (pg/L) in water can be achieved.

Novel aspects:
The solid phase extractionwas optimized for analysis of twelve PFCs in water sample, especially tap water and river water (with low matrix).

No.100387

# Analytical method and homolog distribution of OH-PCBs in ambient Air

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Keywords: OH-PCBs, air, GC/MS

This analytical method is suitable for the determination of Hydroxylated Polychlorinated Biphenyls (OH-PCBs) in ambient air by gas chromatography- high-resolution mass spectrometer with selected-ion monitoring (GC/HRMS-SIM). OH-PCBs are considered as one of the endocrine disturbing chemicals because OH-PCBs have negative impacts on the some thyroid and female hormones. OH-PCBs are formed by metabolism of PCBs by the cytochrome P450 enzyme-mediated oxidation and OH radical reaction of PCBs. The concentrations of OH-PCBs in the environmental waters, air, sediments and living things have been investigated and OH-PCBs have been detected from every medium. In Canada, OH-PCBs were also detected from the rain and snow. In this study, we modified the analytical method and investigated the concentration of OH-PCBs in ambient air. The air was introduced into quartz fiber filter (QFF) and poly urethane foam (PUF) which were set to Hi-Volume air sampler at a flow rate of 700 L/min for 24 hr (total volume was 1000 $m^3$). After collection was completed, clean-up spike (OH-Di-Hp-CB-$^{13}C_{12}$ 3.0ng)was added to the PUF. Both QFF and PUF were simultaneously extracted with acetone by ASE. The acetone extract was added 6 mL of 5% sodium chloride solution, and extracted with 2 mL of hexane, twice. The hexane phase was clean upped with pre-washed Sep-Pak Plus Florisil. Sep-Pak Plus Florisil was eluted with 0.5% diethyl ether/hexane 8 mL (for PCB), and then with 50% acetone/methanol 10 mL (for OH-PCBs). The eluate was concentrated until just before dryness. After derivatization and alkaline digestion, added 6 mL of 5% sodium chloride solution, and extracted with 2 mL of hexane, twice. Concentrated to about 1 mL and dehydrated with anhydrous sodium sulfate. The concentrate was applied to a pre-washed Sep-Pak Plus Florisil, and eluted with 8 mL of 5% diethyl ether/hexane. The eluate was concentrated to100 μL and added syringe spike (MBP-70-$^{13}C_{12}$ 0.50 ng). The method detection limits (MDL) and the method quantification limit (MQL) were 0.067 - 0.13 and 0.17 - 0.33 pg/$m^3$, respectively. The average of recoveries (n = 3) from 1000 $m^3$ of air sample added with 3.0ng OH-PCBs were almost 60-110%, and the relative standard division was 1.2 - 24% . Lowly chlorinated OH-PCBs tend to be collected in PUF and highly ones tend to be collected in QFF. In the air samples, OH-PCBs were detected 1pg/$m^3$ order and the lowly chlorinated OH-PCBs were dominant.

Novel aspects:
OH-PCBs in ambient air was detected using QFF and PUF which were set to Hi-Volume air sampler.

No.100390

# Application of combined UPLC-TOF-MS and combustion ion chromatography for electrolytic degradation mechanism of PFOS in water

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Keywords: Activated-carbon, bond cleavage, byproducts, TOF-mass

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the most extensively investigated representative compounds of perfluoroalkyl carboxylate (PFAC) and perfluoroalkyl sulfonate (PFAS) groups respectively. PFOA can be degraded by UV photodegradation in water while PFOS is extremely resistant to the degradation. But the latter in water can be degraded very easily by electrolytic method than by UV photodegradation. However, electrolytic degradation mechanism and pathways for PFOS are not still known.

Mechanism of electrolytic degradation of PFOS in water is investigated and discussed for the first time using ultra-pressure liquid chromatography time of flight mass spectrometry (UPLC-TOF-MS) combined with combustion ion chromatography. Batch tests for electrolytic degradation (current = 1.0 A, DC voltage: 33.0 V, surface area of circular platinum electrodes = 236 mm2, current density = 4.23 A/m2) of PFOS (concentration = 1.0 mg/L, volume = 250 ml) were conducted using a cylindrical air-tight and heat-resistant glass vessel for 60 min durations. Sodium bicarbonate (50.0 mmol/L) was added to the reaction solution for enhancing electrical conductivity. Liquid samples were collected and analyzed (UPLC-TOF-MS) for PFOS and its degradation intermediates. Head space gas samples were adsorbed to activated carbon columns by continuous nitrogen gas flow through the space. Total fluoride ion concentration and short carbon-chain intermediates in the sample (i.e. activated carbon after head space gas adsorption) were analyzed using combustion ion chromatography. PFOS degradation mechanism in water is discussed based on the identified intermediates.

Though it has already been demonstrated that fluorinated short carbon-chain byproducts of PFOA degradation remain mostly in gas phase (i.e. head-space gas), no such products of PFOS electrolytic degradation in activated carbon columns were observed in this investigation. Absence of fluorinated short carbon-chain compounds and additional fluoride ion contents due to oxidation of the compounds during combustion ion chromatographic analysis strongly suggested that either the compounds were actually absent during PFOS degradation or the compounds in gas phase were not adsorbed to activated carbon. This point still remains to be elucidated. Analysis of water samples by UPLC-TOF-MS showed fragments (with seven carbon atoms) of PFOS having hydroxyl functional group suggesting carbon-carbon bond cleavage adjacent to sulfonate functional group followed by its hydrolysis during electrolytic degradation of PFOS. The present investigation needs to be continued further to understand whether fluorinated short-carbon chain byproducts of PFOS electrolytic degradation do not really exist in head-space gas and elucidate on complete degradation mechanism.

Novel aspects:
Combined UPLC-TOF-MS and combustion ion chromatography technique used for first time in elucidating electrolytic degradation mechanism of PFOS.Carbon-carbon bond cleavage adjacent to sulfonate functional group is the first reaction step.

No.100417

# 2,3,7,8-tetrachlorodibenzo-p-dioxin congener in breast milk among 3 hot spots in Vietnam

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Keywords: TCDD, dioxin congener, hot spot, Vietnam

Using GC-MS to quantify 7 congener of polychlorinateddibenzo-p-dioxins (PCDDs) and 10 congener of polychlorinated dibenzo-p-furans(PCDFs) in breast milk of Vietnamese primiparous mothers who are living in dioxin-hot spots in Vietnam. These areas are former United State Airbases in Southern Vietnam, where herbicide was stored during the Vietnam War. These airbases are knowns as hot spots because of extremely higher level of dioxin in comparison with sprayed and non-sprayed areas. There major hot spots are Bien Hoa, Da Nang and Phu Cat. 52 breast milk samples in Bien Hoa (BH), 43 in Da Nang (DN), and 23 in Phu Cat (PC), and 19 in Kim Bang (KB)- a non-exposed area were collected between 2008 and 2010. Mean total toxicity equivalence (TEQ-WHO 2005) of PCDDs/PCDFs in BH, DN, PC were 10.9, 16.0, 14.8 pg-TEQ/g lipid, respectively while it was 4.51 pg-TEQ/g lipid in KB. Mean of TCDD concentration in BH, DN, PC and KB were 3.60, 2.47,1.79 and 0.64 pg/g lipid, respectively. DN and PC have same congener dioxin and furan profile but different from BH. In DN and PC the relative abundance of 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD) is about 2-3 times higher than 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); however relative abundance of TCDD is higher than that of PeCDD in BH. When TCDD concentration were categorized, percentage of samples have TCDD in the highest category (>10 pg/g lipid) or lowest category (<1 pg/g lipid) in BH are higher than PC and DN, which mean that there is a large variation in TCDD level from BH, especially a small number of mothers in BH are highly exposed. Mean percentage of TCDD contribution to TEQ in BH, DN, PC and KB were 24.6%, 15.1%,12.2% and 13.3%, respectively. Various herbicides were used during the Vietnam War including Agent Orange, Agent Purple, Agent White, Agent Blue, Agent Pink.These herbicides were contaminated with different concentration of TCDD, which may explain the different in TCDD levels from those hot spots.

Novel aspects:
Investigating dioxin and furan congener profile in 3 dioxin - contaminated areas in VIetnam, which are known as hot spots, we found TCDD contribution were different in these hot spots.

No.100428

# The Development of Multi Elemental Analysis of Ferromanganese Nodule by LA-ICP-MS

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Keywords: LA-ICP-MS, Ferromanganese nodule, Standards, Multi elemental analysis

## Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a useful method for determination of trace elements distributions. It shows higher sensitivity than EPMA or SEM-EDX which are also employed for similar purposes. Furthermore, it is possible to observe samples under atmospheric pressure by LA, thereby protecting samples against structural changes. However, the drawbacks of LA-ICP-MS are obtaining. Matched standards as established standards do not exist. Secondly, it is difficult to obtain stable signals and accurate determination values by LA-ICP-MS compared with ICP-MS. The data obtained is more accurate when subjected to internal standard correction and selection of internal standard for the element of interest is to be carefully considered paying attention to the features of the sample. Therefore, the purpose of this study is to obtain accurate determination values by LA-ICP-MS.

Multi elements in ferromanganese nodules, which are one of the notable and important geological samples, were determined by LA-ICP-MS and the data were compared with those of ICP-MS in this work. Certified reference material (CRM) of ferromanganese nodule and manganese dioxide ($MnO_2$) were used to prepare matrix matched standards. Magnesium which is one of the major elements in ferromanganese nodules was selected for internal standard correction.

## Sample preparation

JMn1 which is a CRM of ferromanganese nodule collected in the Pacific Ocean (purchased from AIST) and high purity $MnO_2$ powder (99.5%, Wako Chemical Co.) were used for preparation of standards for LA-ICP-MS. JMn1 and $MnO_2$ with the weight ratios of 0, 25, 50, 75, 100% were mixed. These samples were pressed into $\phi$5 mm pellets for IR analysis. Ferromanganese nodule collected in Pacific Ocean near Hawaii (10 ° 10'N, 147 ° 00'W, JOGMEC) was cut across the center, and the obtained powder was collected and made into pellets using the same procedure as standards. These pellets and powder are mentioned as Hawaii samples in this paper.

## Instrumentation and parameters

UP213 (NEW WAVE), LA instrument and ELEMENT XR (Thermo Fisher) ICP-MS instruments were used. The conditions of LA are as follows: 213 nm wavelength, 100 $\mu$m diameter, 10 Hz repetition rate, 15 $\mu$m/sec scan speed, 0.023 mJ pulse energy, and raster sample scan mode. The conditions for ICP-MS were 1.23 L/min sample gas and middle resolution. The elements measured were $^{11}$B, $^{24}$Mg, $^{52}$Cr, $^{56}$Fe, $^{60}$Ni, $^{63}$Cu, $^{95}$Mo, $^{111}$Cd, $^{140}$Ce, $^{153}$Eu, $^{175}$Lu, $^{208}$Pb, and $^{238}$U.

## Results and discussion

Calibration curves for LA-ICP-MS measurements were successfully obtained. The square of correlation coefficient values ($R^2$) of the calibration curves were 0.932~0.984. $R^2$ values of the calibration curves obtained by internal standard correction using $^{24}$Mg, which contained 4 points except for blank, were 0.924~0.999. Good linear calibration curves were obtained with internal standard corrections. For example, $R^2$ value of $^{56}$Fe without correction was 0.978, and correction with $^{24}$Mg was 0.991. Calibration curves of $^{52}$Cr and $^{111}$Cd were not obtained due to its low ablation efficiency due to their chemical property or low isotopic abundances.

Accuracy is defined as $[C_{LA\text{-}ICP\text{-}MS}] / [C_{ICP\text{-}MS}] \times 100$ in this study. $[C_{LA\text{-}ICP\text{-}MS}]$ and $[C_{ICP\text{-}MS}]$ are the determination values of the Hawaii sample obtained by LA-ICP-MS and ICP-MS respectively. The accuracy obtained without internal standard correction were in the range 97.4~173%. That with internal standard correction using $^{24}$Mg were 80.4~110%. Internal standard correction showed accurate determination values. For example, the accuracy of $^{56}$Fe without correction was 135% and with internal standard correction using $^{24}$Mg was 110%. Thus, accurate determination values were obtained by this method.

Novel aspects:

Multi elements in ferromanganese nodules were determined by LA-ICP-MS and the data were compared with those of ICP-MS in order to obtain accurate determination values by LA-ICP-MS.

No.100446

# Profiling waters from natural sources and areas of oil sands activity using Fourier transform ion cyclotron resonance mass spectrometry

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Keywords: Fourier transform ion cyclotron resonance mass spectrometry, atmospheric pressure photoionization, environment, oil sands

Due to pressures on a finite supply of petroleum as consumption continues to grow, it is necessary to turn to less conventional sources, such as oil sands. The Athabasca oil sands are located in Alberta, Canada, and consist of clay, sand, water, and bitumen. An alkaline hot water extraction process can be used to separate the bitumen, which can then be upgraded to synthetic oil. Approximately three barrels of water are consumed during the production of one barrel of oil, resulting in intensive water usage by the oil sands industry. This oil sands process water (OSPW) must be stored in vast tailings ponds, as there is a zero discharge policy, and there is a need to monitor potential effects upon local water quality. It is important to be able to differentiate between those organic components found in the aquatic environment due to natural processes, such as expected seepage of oil sands material, and those which arise due to human activity.

Samples were acquired from a range of natural water and oil sands process water sites in the Athabasca Basin and analysis was performed using a 12 T solariX Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Previous research into the characterization of OSPW has typically relied upon the usage of electrospray ionization (ESI), usually due to the targetting of naphthenic acids within the complex mixtures. Whilst ESI is best suited to the study of polar and ionic species, atmospheric pressure photoionization (APPI) can be applied to the study of less polar species and can produce radical ions in addition to protonated/deprotonated ions. ESI and APPI represent complementary methods, where APPI is an amenable ionization method for the study of a broad range of compounds, such as less polar, sulfur-containing compounds and hydrocarbons which do not incorporate heteroatoms. Mass spectra of complex mixtures that have been acquired using APPI are typically more complex, due to the greater number of components observed and the fact that radical ions are observed in addition. High field FTICR mass spectrometry offers ultra-high resolving power and mass accuracy, which afford high confidence in assignments of species within complex mixtures, which is particularly important for mass spectra generated using APPI.

Following assignments of elemental compositions, it is possible to visualize the data using different methods of categorization, such as heteroatom content, carbon number, "hydrogen deficiency" (Z), or double bond equivalents (DBE). Principal component analysis (PCA) has been used to highlight similarities and differences between water sources. Differences were found between river waters, groundwaters, and OSPW, indicating potential for determining anthropogenic influences on the aquatic environment.

Novel aspects:
Usage of APPI and FTICR mass spectrometry for the characterization and comparison of organic components, including compounds of low polarity, found in waters from different sources in the Athabasca region

No.100452

# The Development of Analytical Method for Speciation of Transition Metal Ions in Seawater by CE-ESI-MS

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Keywords: CE-MS, transition metals, speciation, seawater

## Introduction

It is difficult to determine concentration of transition metals dissolved in seawater. Acquisition of their dissolution states is more difficult than their determination. In order to characterize transition metal ions in seawater, a new analytical system should be required. ESI-MS (Electrospray Ionization Mass Spectrometry) is known to be one of the soft ionization methods, and many reports on the dissolution states have been presented. However, detection of these dissolution states in seawater is difficult as well as avoiding the "ion-suppression effect". A high concentration matrix (sodium ion) suppresses detection of trace metal ions. To resolve this problem, pretreatment is required to separate trace metal ions from high concentrations of matrices while keeping dissolution states of original metal ions. In this study, capillary electrophoresis (CE) was used as a separation technique, and online hyphenated CE(-ESI)-MS was developed. The separation method with CE is simple, while it reasonably maintains the dissolution states of objective metals, compared with other separation methods. For CE-MS analysis, volatile background electrolytes (BGEs) are required to obtain high sensitivity. In this study, non-volatile hydrochloric acid (HCl) was used as the BGE because volatile BGEs, such as formic or acetic acids, easily coordinate with transition metal ions. Manganese (II) and Cobalt (II), which have analogous properties, were measured in high concentrations of NaCl solutions by CE-MS.

## Application

CE-MS is composed of online hyphenation of CAPI-3300 (Photal) and LC-MS 2010A (Shimadzu) with self-designed tri-axial ESI interface. Separation voltage (+30kV) was applied by CAPI-3300, and ionization voltage as +4.5kV by LC-MS 2010A. Ultrapure water (18.2MΩ) was used as sheath liquid with 0.01 mL/min flow rate. The uncoated separation capillary (I.D. 70 μm, length 110cm) was used.

## Experimantal

All sample reagents (Kanto Chemical Co) used are of special grade. 1) Solutions containing 0.1mM of manganese chloride ($MnCl_2$) or cobalt chloride ($CoCl_2$) were measured by ESI-MS. 2) Solution containing 5mM of $MnCl_2$, $CoCl_2$ and 500mM of NaCl was measured by CE-MS with 1mM of HCl as BGE. The concentration of 500mM NaCl is almost the same as that of seawater. Conditions of sample injection to separation capillary were examined (5kPa for 5~120 sec).

## Results and Discussion

### 1) Measuring solutions of $MnCl_2$ and $CoCl_2$ with ESI-MS

When an aliquot of $MnCl_2$ solution was measured by ESI-MS, $[Mn(OH)(H_2O)_{0\sim5}]^+$, $[MnCl(H_2O)_{3\sim4}]^+$, and $[Mn(H_2O)_{3\sim5}]^{2+}$ were observed. It is previously reported that $[M(OH)(H_2O)]^+$ (M: Metal) is derived from hydrolysis of $[M(H_2O)_2]^{2+}$ at liquid-phase and gas-phase in ESI-MS. When $CoCl_2$ solution was measured, $[Co(OH)(H_2O)_{0\sim5}]^+$, $[CoCl(OH)_{3\sim4}]^+$ were also observed but $[Co(H_2O)_n]^{2+}$ was not observed. Cobalt ion is more easily hydrolyzed than manganese ion. Thus, accurate information on sample solutions could be obtained by ESI-MS.

### 2) Measuring solutions of $MnCl_2$ and $CoCl_2$ in high concentration of NaCl with CE-MS

First, amounts of sample solution for the capillary were examined. When injection times of sample solution were 30~60 sec, sharp and high peaks of objective metal species were observed on the electroferrogram. When samples contained extremely high concentration of components, stacking of minor component occurred in the CE system (Self-stacking). When injection time of sample solution was over 80 sec, cluster ions of NaCl (m/z 81+58n) were also stacked and led to ion-suppression effect. Therefore, the optimal condition of sample injection was 5 kPa for 30~60 sec. Observed metal species with CE-MS were good accordance with ESI-MS. Thus, the separation of trace metal ions from high concentration of NaCl in keeping with the dissolution states of the original metal ions by CE-MS was accomplished.

Novel aspects:

For seawater analysis, the separation of trace metal ions from high concentration of NaCl in keeping with the dissolution states of the original metal ions by CE-MS was accomplished.

No.100462

# Analysis of biomarkers of the pesticide imazalil using LC/MS/MS.

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Keywords: Biomarkers, Pesticides, LC/MS/MS, Quatification, Urine

Introduction - Imazalil is a widely used post-harvestfungicide applied on a variety of crops, especially on fruits. Imazalil is alsoused in veterinary medicine as an antimycotic drug.
Imazalil is classified as "Likely to be carcinogenic inhumans," according to EPA. Exposureto imidazole derivatives is also suspected to produce craniofacialmalformations in vertebrate development. Studies on rats have shown thatimazalil is metabolized into 25 different metabolites with 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)-1-ethanol (DCPI) asthe major metabolite. There are very fewstudies in humans, however a case study of a patient treated with imazalil indicateda half-time of 2 h in serum. Various methods have been published for determinationof imazalil residues in agricultural and food products. However, no methodshave been described for determination of imazalil or its metabolites in humanbiological samples. Human occupational exposure can be substantial especiallyin developing countries were the use is high. It is especially common thatwomen in fertile age are exposed during post-harvest treatment. Thus, methodsfor measurement of biomarkers of exposure is valuable. The aim of this studywas to develop a simple liquid chromatography-tandem mass
spectrometry (LC/MS/MS) method for the analysis of imazaliland DCPI in human urine.
Method Several SPE columns were evaluated and optimized forsample pretreatment. The eluates were analyzed using electrospray ionization and selected reaction monitoring(SRM) in positive ion mode after separation on a C18 LC column. The mobile phase used was water and methanol with0.1% formic acid. $D_5$-labeled imazalil was used as an internalstandard.
Results Two SPE columns could be used for samplespretreatment, C2 and Oasis HLB. The sample was retained at neutral pH andeluted using 1% formic acid in methanol. For quantification of imazalil the transition atm/z 297.1-159.0 and for DCPI the transition m/z 257.0-69.2 was used. The method have an LOD of 1 ng/ml for both imazaliland DCPI. Data on levels found in a population will be presented.

Novel aspects:
This study presents a new quantitative method for quantification of the pesticide imazalil and its major metabolite DCPI in human urine using LC/MS/MS.

No.100486

# Mass spectrometric investigation of mechanisms for methane formation from epicuticular wax under aerobiosis and UV

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Keywords: GCMS, photolysis, methane , greenhouse gases

## Introduction

Methane ($CH_4$) is the second most important long lived greenhouse gas. The total global $CH_4$ source strength is well described, whereas substantial uncertainties exist about the strength of individual source components. When it was discovered that terrestrial plants emit $CH_4$ under aerobic conditions it presented quite a conundrum as $CH_4$ is a highly reduced molecule. The ubiquitous plant structural component pectin is a highly activated methyl donor, and in purified form it emits $CH_4$ in response to UV-radiation. Therefore, pectin is regarded as one of the most likely precursors in plants to aerobically emitted $CH_4$. *In vivo*, however, pectin is situated under the cuticle and in between primary cell walls and is very well protected against UV-radiation due to absorption and reflection by the outer epicuticular wax layers. This raises the question to what extent UV-induced $CH_4$ production may occur at the plant surface wax layer. In this presentation we report the mass spectrometric investigations of the photolytic pathways.

## Materials and methods

Seeds of *Brassica oleracea capitata f. alba*, donated by Nordic Gene Bank, were germinated in potted soil and grown for seven weeks in a climate controlled chamber. The *B. oleracea* surface wax was gently removed and exposed to 17 W $m^{-2}$ of UV-B (309-314 nm) for 331 h using Philips PL-S 9W/01 2 P 1 CT tubes while incubated in gas tight, UV transparent glass vials.

*Analysis*

Head space aliquots of 500 µl were sampled and identification of the gaseous products ($C_1$-$C_3$) was performed with a Varian 3400 gas chromatograph interfaced to a Saturn II ion trap mass spectrometer (GCMS). The compounds were separated using a PORAPLOT U fused silica column

The leaf surface wax was analyzed by GCMS using a Hewlett Packard HP 6890 gas chromatograph interfaced to a HP5973 mass selective detector. The products were separated using WCOT fused silica column coated with VF-23 and identified using NIST search engine, version of 2.0 f.

UU/VIS spectra were obtained using a HPLC system and a UV6000LP PDA-detector

## Results and discussion

A trial resulted in specific $CH_4$ emission rate of 153 – 14 nmol $CH_4$ $g^{-1}$ wax $h^{-1}$ in air when exposed to UV-radiation. The GCMS analysis of the leaf wax revealed ($C_{29}H_{60}$) and 15-nonacosanone ($C_{29}H_{58}O$) as the predominant compounds accompanied by linear C13-C15 aldehydes, 2-pentadecanone and 2-hexadecanone in low concentrations. The surface lipids have only a weak UV absorption tailing into the 300-330 nm region. However, the photolytic products of isolated surface wax as well as the secondary products were identified by GCMS analysis.

In sum, methane formation from leaf surface wax is a two step process initiated by a photolytic rearrangement reaction of the major wax component, i.e. 15-nonacosanone followed by an α-cleavage of the generated ketone. In the latter reaction the presence of concurrent path ways become significant for the yield of methane as only the Norrish I route leads to methane. The ratio of the Norrish I and II routes can be estimated using the two products 1-tridecene (Norrish II) and tetradecane (Norrish I). This ratio $C_{14}H_{30}/C_{13}H_{26}$ has been estimated to 0,021, and hence the methane route is a minor, but nevertheless significant process.

Mass spectrometry was found to be an excellent analytical tool for rationalization of the photolytic pathways

Novel aspects:
The combination of GCMS analyses snd photolytical studies has resulted in detailed understannding of the underlying mechanisms for aerobic formation of methane from plants.

No.100532

# Application of HPLC/MS in determination of heroin metabolites in saliva an urine samples

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Keywords: saliva; urine; heroin; metabolite; glucuronide

## Introduction

Urine is a common, and saliva represents an alternative specimen for substances of abuse determination in toxicology. In this study, one step was to optimize a method for saliva and urine specimen preparation for heroin metabolites, codeine, 6-monoacetylmorphine (6-mam) and morphine determination by high performance liquid chromatography-mass spectrometry (HPLC/MS), and then this method was applied on saliva and urine samples taken from the patients. As a preliminary analysis, test strips for opiates identification in patients‘ urine were used. Saliva and urine samples from patients whose preliminary test was "positive" were taken for further analysis.

## Methods

*Sample preparation*

Both, saliva and urine specimens was prepared using liquid/liquid extraction of codeine, 6-mam and morphineby mixture of chloroform and isopropanol (9:1; v/v).

*HPLCMS analysis*

Extracts were analyzed by HPLC-ESI-MS technique: at Waters Alliance fi system, the separation column Waters Spherisorbfi 5 μm, ODS2, 4.6 × 100 mm was used; mobile phase: ammonium acetate : acetonitrile (80:20; v/v), mobile phase flow rate 0.3 mL/min, autosampler temperature 20 ° C; injection volume 50 μL;Mass detection range: 100400 m/z, centroid mode, interscan delay 0.1 s, scantime0.5 s, splitless, four voltage values: 70, 60, 50 i 38 V, ES+, source temperature 150 ° C , desolvation temperature 430 ° C, gas flow for desolvation 362 L/h and at cone 135 L/h, capillary voltage 3 kV - mass spectrometer Waters Micromassfi ZQ™ (Waters Corporation, Milford, MA, USA).

Calibration and optimization were done using morphine standard (ion 286) 10 mg/L at flow rate 10 mL/min.Regression and correlation analyses were performed with the probability level of 0.05. Mass spectra were analyzed by software Waters MassLynx™ (Waters Corporation, Milford, MA, USA).

## Results and Discussion

Calibrations for each analyzed substance in both specimens were done in the concentration range from 0.1 to 1 mg/L and the coefficients of correlation wereabove 0.99. Recoveries for morphine and codeine determination in saliva was 99%, while for 6- mam it was 94% and recoveries for morphine, codeine and 6-mam for determination in urine were 103%, 101% and 93%, respectively. Limits of detection and quantification of a proposed method were 0.01 mg/L and 0.05 mg/L, respectively for both sample types.

Applying the proposed method on real samples of patients (n=10) where positive reaction was obtained on test strips we determined concentrations of heroin metabolites in selected samples in following ranges: Concentration of codeine in the saliva of the heroin abusers ranged from 0.05 to 5.33, for morphine between 0.05 and 5.33 and for 6-mam between 0.01 and 0.68 mg/L.Concentration of codeine in the urine samples of the same patients ranged from 0.22to 5.74, for morphine between 0.15 and 6.32 and for 6-mam between 0.05 and1.78 mg/L.

A proposed HPLC/MS method for codeine, 6-mam andmorphine determination in saliva and urine samples is accurate, simple, cheap and suitable for routine analysis and monitoring of heroin abuse.

Novel aspects:

Developing accurate, simple, cheap and suitable method in determination of heroin metabolites in saliva an urine with HPLC/MS

No.100618

# Analysis method of polybrominateddiphenylether using GC-MS and GC-MS/MS coupled with automated identification and quantification system with a database

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Keywords: GC/MS, GC-MS/MS, polybrominateddiphenylether

There are 209 isomers of polybrominated diphenyl ether (BDEs) with each one showing different level of toxicity and detection frequency in environmental samples. GC-MS is commonly used for the analysis of BDEs.

A common method of BDEs analysis requires the expensive standards and instrument of gas chromatograph/ double focusing high resolution magnetic sector mass spectrometer (GC-HRMS). Moreover, the handling and maintenance of GC-HRMS instruments is a time-consuming and labor-intensive work. This situation is critical problem especially for environmental laboratories in under developing countries.

Kadokami *et al.* [1] developed a novel automated identification and semi-quantification system with a database (AIQS-DB) which allows an automatic identification and semi-quantitation of 1,000 pollutants without standard sample analysis. The database includes retention indices, mass spectra, and internal calibration curves for 1,000 pollutants. The pollutants are identified using the mass spectrum and retention time predicted by retention index and retention times of *n* -alkanes. Semi-quantitation is performed using internal calibration curve.

We developed a method for BDEs analysis. For reduction of the necessary authentic standard, AIQS-DB was applied to BDEs with less toxicity and detection frequency, while the conventional method (isotope dilution method) was applied to BDEs with a higher toxicity and detection frequency in order to obtain precise quantitation results. For easy handling and maintenance, single quadrupole mass spectrometer of GC-MS was used.

The developed method was applied to sediment samples. BDEs were detected and semi-quantitation results were obtained. However, several samples with heavy matrix could not detected BDEs due to peak overlapping. GC-MS was not applied to heavy matrix sample analysis such as sediment sample. Gas chromatographytriple quadrupole mass spectrometry (GC-MS/MS) was applied to improve the separation of BDEs from heavy matrix, ensuring easy handling and maintenance. Using GC-MS/MS, BDEs were successfully identified and determined, corresponding to the results by the conventional method by HR-GCMS.

1. Novel gas chromatography-mass spectrometry database for automatic identification and quantification of micropollutants, Kiwao Kadokami, Kyoko Tanada, Katsuyuki Taneda, Katsyhiro Nakagawa, *J. Chromatogr A*, 1089 pp 219-226, 2005.

Novel aspects:

For analysis of BDEs using GC/MS or GC-MS/MS, the novel method using AIQS-DB and the conventional (isotope dilution method) was developed for easy operation and reduction of standards.

No.100653

# LC-HRMS investigation of the human in vitro metabolism of brominated flame retardants using APPI and ESI.

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Keywords: Brominated flame retardants, APPI, High resolution mass spectrometry, metabolism

Brominated flame retardants (BFRs) are widely used in the production of polymers and plastics in order to prevent fires in electronic and domestic goods. These compounds, mainly represented by tetrabromobisphenol-A (TBBPA) and polybromodiphenylethers (PBDEs), are suspected to act as endocrine disruptors [1,2] and their toxicity is of growing concern. However, although the occurrence of BFRs has been shown in almost all environmental compartments, limited data is available about their fate, in particular in humans.

GC-MS is widely used nowadays for the analysis of PBDEs, though not perfectly suited for the highest MW PBDEs (octa- to deca-BDEs), mainly due to thermal degradation problems. For TBBPA, a derivatisation step is required. In this work, LC-MS methods based on ESI and APPI associated with high resolution ion analysis, were developed for the analysis of BFRs metabolites, which we expected to be moderately polar to polar molecules. These methods were applied to the study of *in vitro* metabolism of BFRs after incubation with human primary cultures of hepatocytes. HRMS full scan analysis was found to be useful not only for the identification of known metabolites, but also for the detection of non targeted metabolites, which were identified by MS/MS experiments.

First, the efficiency of ESI, APCI and APPI was compared for the analysis of TBBPA, PBDEs and their metabolites formed after incubation with hepatocytes. ESI was found to be well suited for the analysis of TBBPA and their metabolites, as well as for hydroxylated metabolites of PBDEs. We have previously shown that LC-MS using Atmospheric Pressure Photo Ionization (APPI) could constitute a possible alternative for the analysis of PBDEs [3]. In the continuation of our work, the use of APPI was assessed for the analysis of PBDE metabolites with particular emphasis on hydroxylated PBDEs.

After the optimization of APPI ionization parameters (nature and % of dopant, heated nebulizer and transfer capillary temperatures, LC mobile phase composition and flow rate), a high resolution acquisition was set up on a LTQ-Orbitrap instrument operating in the full scan mode at a resolution of 60,000, which was necessary to get a total resolution of *e.g.* the m/z 498,7 ions formed from both OH-tetra-BDE $[M-H]^-$ ions and OH-penta-BDE $[M-H-HBr]^-$ ions.

The developed methods were then applied to the study of TBBPA and BDE-47 metabolism after *in vitro* incubations with human hepatocytes. Absolute quantification of metabolites was achieved by using radiolabelled molecules. LC-ESI-HRMS allowed the identification of conjugated forms of TBBPA, namely glucuronide, sulphate and doubly conjugated metabolites. For BDE-47, LC-APPI-HRMS allowed not only the identification of expected metabolites such as hydroxylated PBDEs, but also the characterization of other metabolites, such as a tetra-BDE dihydrodiol and conjugated metabolites. For the latter metabolites, identification was completed by the use of ESI, allowing the access to the m/z of quasi-molecular ions.

Results obtained *in vitro* using human primary hepatocyte cultures as well as other human cell lines demonstrate that human cells can biotransform TBBPA into conjugated metabolites and BDE-47 into hydroxylated, dihydrodiol and conjugated metabolites. The potential of these metabolites to be used as biomarkers of exposure in human samples will further be evaluated within the frame of ongoing impregnation studies.

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Novel aspects:

Complementary use of ESI and APPI for complete characterisation of BFRs formed in vitro. HRMS provides discrimination between isobaric hydroxy-polybrominated diphenylethers. New information about metabolic fate of BFRs in human.

No.100659

# Information of the behavior of the persistent organic pollutants in the sea around Japan

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Keywords: POPs, HCHs, enantioselective analysis, Japan Sea

Although a large amount of Persistent Organic Pollutants (POPs) have been used in Asian countries such as China or Korea, the state of contamination has not been clarified in Japan Sea surrounded by these countries. In this study, pollution levels of POPs in sea water and air over the ocean were investigated by using voluntary sampling of passenger ships. Also isomer and enantiomer analysis were conducted to estimate the source and pollutant pathway of POPs.

The sea water samples (approx. 50L) and air samples(approx.108m$^3$) were taken by the passenger ship (NYK cruses CO., LTD , ASKA-2) equipped with concentrating device between 2009 and 2011.Also in downstream site of Chang Jiang, water samples(approx.6L) were taken in 2010 and 2011.

Identification and quantification of POPs were performed using a gas chromatograph, (HP6890N Agilent) /high-resolution mass spectrometer(800D,JEOL Ltd.) equipped with HT-8PCB capillary column (60m*0.25mm id.Kanto Chemical) and BGB172 capillary column(30m*0.25mm id,BGB Analytik AG) for enantioselective analysis

In this result, the highest concentration of Hexachlorocyclohexanes (HCHs) was in the sea around the northern Hokkaido,880pg/L. DDTs was higher in Tsushima straits than in the north of Japan Sea. On the other hand, it is several times higher concentration in Chang Jiang than in around the Sea of Japan, 1300～2600pg/L (HCH).

From the view point of isomer pattern, the ratios ( $\alpha$ / $\gamma$ ) of Sea waters were apparently different according to the areas. In general, lower $\alpha$ / $\gamma$ ratios were observed in low-latitude region. Especially in downstream site of Chang Jiang, it was the lowest value,1.4–0.1, and 1.9–0.6 in the seas around Korea,

4.2–0.6 in southwestern Sakhalin. These results seem to reflect the usage of $\gamma$ -HCH(Lindane) in China and indicate that HCHs was discharged to the marginal seas of the region through rivers in considerable amounts and transported to Japan Sea via oceans.

Dechlorane Plus (DP) is a chlorinated FR additive introduced as a replacement for Dechlorane, or Mirex.

In the past, there are few data of DP in the environment. In this study the concentration of DP in the sea water was investigated for the first time. The range of DP is 0.7～14pg/L in the sea around Japan and 5～16pg/L in Chang Jiang.

As for chiral analyis, it is regarded that enantioenrichment indicates that it was released some time ago and has since been subjected to recycling from water or soil. EF value of $\alpha$ -HCH was close to racemic in southwest Sakhalin(0.5–0.02) , downstream site of Chang Jiang (0.51–0.02) . In Chang Jiang and the northern Hokkaido, enantiomer fractions (EFs) of alpha-HCH was nearly 0.5(racemic). It suggests that those areas were affected by relatively-recent pollutant source.

In Japan Sea, as latitude become higher, EF values tend to become lower. Since 2005's survey , EF values was 0.46–0.05 in middle Japan Sea, 0.44–0.03 in northern Japan Sea( around Hokkaido),0.46–0.01(pacific sideboard of Tohoku area). These results indicate that southwest Sakhalin, downstream site of Chang Jiang and Tsushima Straits area polluted by relatively new HCHs.

From comprehensive viewpoint, there is a possibility of contamination in Japan Sea, which is caused by the transportation from other countries and polluted area by HCH was found in around Hokkaido.

Novel aspects:

Pollution levels of POPs in sea water and air over the ocean were investigated. Also isomer and enantiomer analysis were conducted to estimate the source and pollutant pathway of POPs.

No.100689

# LC/ESI(-)-MS/MS analyses of the biotransformation products of dibenzo-p-dioxin by Sphingobium sp. strain KK2

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Keywords: LC/ESI(-)-MS/MS, dibenzo-p-dioxin, bacteria

Biaryl ether environmental pollutants such as dibenzo-*p*-dioxin, dibenzofuran and their halogenated congeners are considered to be environmentally-persistent pollutants that originate from incineration processes of domestic and industrial waste. There is much interest to know the ultimate environmental fates of these compounds and this includes the contributions of soil microorganisms to their biotransformation. A soil bacterium, *Sphingobium* sp. strain KK22 was recently isolated from a hydrocarbon-degrading microbial consortium and was found to biotransform dibenzo-*p*-dioxin following induction on the 3-ring polycyclic aromatic hydrocarbon phenanthrene. Strain KK22 was exposed to 50 mg/L dibenzo-*p*-dioxin in 50-ml size microcosms and was sampled multiple times over 48 hours. Liquid chromatography electrospray ionization tandem mass spectrometry coupled with UV detection in negative ionization mode (UV-LC/ESI(-)-MS/MS) was conducted by full scan analyses on acidified microcosm organic extracts and revealed multiple putative ions of interest that were not detected in biotic and abiotic controls. Further investigation by CID product ion scanning showed that least two biotransformation products corresponding to the deprotonated molecular ions $[M - H]^-$ = 251 and $[M - H]^-$ = 267 were present. Fragmentation analyses revealed that these compounds appeared to be ring-opened structures derived from initial angular dioxygenation of the dibenzo-*p*-dioxin molecule by strain KK22 and they were tentatively identified as 6-(2-hydroxyphenoxy)-6-oxo-hex-4-enoic acid and a singly-hydroxylated derivative respectively. If confirmed, these chemicals shall represent previously unreported metabolites from dibenzo-*p*-dioxinby a bacterium. Further investigation is continuing to determine the nature of dibenzo-*p*-dioxin transformation by strain KK22 through the utilization of LC/ESI(-)-MS/MS.

Novel aspects:
LC/ESI(-)-MS/MS was utilized to determine biotransformation products of dibenzo-p-dioxin by a bacterium.

No.100693

# Analysis of Sea-Dumped Chemical Warfare Agents from Sediment Samples Taken at in Baltic Sea

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Keywords: chemical warfare agents, Baltic sea, environmental analysis, GC-EI/MS, LC-APCI/MS/MS

After the Second World War, a large amount of chemical warfare agents (CWAs) were dumped at the Baltic Sea. For example, in the Bornholm dumpsite located within Danish economic zone, it is estimated that over 500,000 shells and containers containing over 11,000 tons of chemical warfare agents were dumped in 1947 by the Soviet Military Authority in Germany.

In this study, sediment samples were collected during different sampling cruises conducted in the Bornholm dumpsite or around it between 2006 and 2011. The target chemicals were mustard, tabun, Clark I & II, Adamsite, Lewisite I & II and $\alpha$-chloroacetophenone as well as arsenic oil containing Clark I, triphenylarsine, phenyldichloroarsine and trichloroarsine, and related degradation products. After an appropriate sample preparation procedure, the samples were analysed using GC-EI/MS with selected ion monitoring (SIM) as such and after derivatisation with propanethiol, and LC-APCI/MS/MS with selected reaction monitoring (SRM) as such and after oxidation using hydrogen peroxide.
Analysed sediment samples showed considerable spreading of arsenic-containing chemicals within the dumpsite as well as between the dumpsite and the Bornholm Island. The highest found sediment concentrations have been over 16,000 ng/g of degradation products of Clark I and 39,000 ng/gof triphenylarsine within the primary dumpsite. [1]

Baltic Sea contains also unexplored areas, e.g. Gotland and Gdansk deep, that have been claimed as dumping sites of CWAs. The Chemical Munitions Search and Assessment (CHEMSEA) project has been established to carry out investigation at the official and unofficial dumping areas in the Gotland and Gdansk deep. The main focus of the project is to locate the dumped CWAs and sample the surrounding environment to assess the possible threat. The outcomes of the project will also include recommendations on operating procedures, and guidelines to employ in those areas, to be used by maritime administrations of respective countries, as well as a contingency plan for risk management. The CHEMSEA project is a transnational collaboration including project partners from five EU countries and a number of associated organizations, including governmental agencies and international organizations. This project is part of the Baltic Sea Region Program and is partly financed by the European Union. The project was started in the fall of 2011 and will continue until early 2014. [2]

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Novel aspects:
Novel information of CWA contamination of the Baltic sea.

No.100699

# Chiral Chemicals as Tracers of Sources and Fate Processes in a World of Changing Climate

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Keywords: persistent organic pollutants, pesticides, chiral, climate, arctic

Elimination of persistent organic pollutants (POPs) under national and international regulations reduces "primary" emissions, but "secondary" emissions continue from residues deposited in soil, water, ice and vegetation during former years of high usage. In a future, secondary source controlled world, POPs follow the cycle of organic carbon and biogeochemical processes determine their transport, accumulation and fate (1). Climate change is likely to affect mobilisation of POPs through e.g., increased temperature, loss of ice cover in polar regions, melting glaciers and changes in soil microbiology which affect degradation and transformation (2). Chiral compounds offer special advantage for following transport and fate pathways because of their ability to distinguish racemic (newly released or protected from microbial attack) and nonracemic (microbially weathered) sources. Use of GC or LC with chiral stationary phases and MS makes enantiomer-specific analysis possible for chiral compounds of many chemical classes. This paper discusses the rationale for this approach and suggests applications where chiral POPs (3) and other compounds could aid investigation of climate-mediated exchange and degradation processes. Examples include distinguishing agricultural vs. non-agricultural and recently used vs. residual pesticides, degradation and sequestration processes in soil, historical vs. recent atmospheric deposition, sources in arctic air and influence of ice cover on volatilisation.

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2.UNEP/AMAP 2011. *Climate Change and POPs: Predicting the Impacts*. Report of the UNEP/AMAP Expert Group, Secretariat of the Stockholm Convention, Geneva, 62 pp.

3.Bidleman, T.F., Jantunen, L.M., Kurt-Karakus, P.B., Wong, F. 2012. Chiral compounds as tracers of sources and fate: review and prospects for investigating climate change influences. *Atmos. Pollut. Res*., submitted.

Novel aspects:
Chiral compounds offer special advantage for following POPs pathways because of their ability to distinguish emissions from racemic (newly released or protected from microbial attack) and nonracemic (microbially weathered) sources.

No.100746

# Online SPE LC-MSMS for screening and quantifying anti-cancer drugs and metabolites in hospital's wastewaters and rivers

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Keywords: cancer drugs, waste water, LC-MSMS

**Introduction :**

Over 50 cytotoxic chemotherapies are used in hospitals. The main anti-cancer drugs used in cancer chemotherapy can be classified into several categories: cytotoxic, the most represented, but also hormones, immune response modifiers and antibodies. Most cytotoxic agents used in cancer chemotherapy interact with DNA or its precursors. Very few studies are evaluating the future of these drugs in wastewater. Potential risks associated with these discharges are poorly understood and require study work and research to better understand the hazards, exposure characterization and assessment risks to human health and the environment. The purpose of the study is to establish an analytical methodology to screen most of the anti-cancer drugs currently used in hospital waste waters.

**Methods :**

The goal was to develop a simultaneous analysis of a large number of anticancer drugs from water samples with a limited amount of sample. These samples are processed by on-line solid phase extraction (SPE) to isolate and concentrate the different cytotoxics. Following extraction, compounds are transferred to an UHPLC column for separation. Detection is performed using Multiple Reaction Monitoring mode on an ultrafast triple quadrupole mass spectrometer. Special attention was given to the orthogonal selectivity and to the working pressure compatibility of the extraction and analytical columns.

**Preliminary results:**

The UHPLC system (NEXERA, Shimadzu Corp.) is equipped with an online SPE cartridge which, thanks to the innovative switching valves configuration, is isolated from the high pressure LC. This allowed us to combine on-line SPE with UHPLC. The first step of the project was to optimize the MRM transitions for each molecule of the chosen list and to choose the best mobile phase composition compromise for optimum sensitivity. Once that process done for the 25 selected drugs, several SPE sorbent for extraction of a wide range of different chemical class compounds were tested. The same study was conducted on the choice of the analytical column trying to combine several retention mechanisms. The final stage of development of the method was devoted to optimization of the sensitivity to reach the lowest limit of quantification and to study of the dynamic range for each molecule. Finally the methodology was validated with real wastewater sample to assess extraction and ionization recovery as well as ruggedness of the system.

Novel aspects:

The main difficulty is the wide panel of chemical structures to be separated using a single rapid automated method

No.100757

# Identification of chlorinated aromatics as impurity of chlorinated paraffins by GC-HRMS or GC-HR-Tof-MS

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Keywords: POPs, Chlorinated Paraffins, Impurities, Identification, PCB

Chlorinated paraffins (CPs) are industrial products used as metal-working fluids and flame retardants for plastic materials. Short chain CPs (SCCPs) seems to persist in the environment and bioaccumulate in biota, and are under review by the Stockholm Convention on persistent organic pollutants.
We identified high levels of POPs such as PCBs, PCBz, PCNs and HCHs from air sampler materials. After detailed investigations these POPs are concluded as impurities of technical chlorinated paraffins (CPs), which used for rubber materials as flame retardant at 2-6 % contents. The exact mass spectrum and accurate mass of these chemicals are fixed theoretical mass with few ppm errors. The profile of PCBs congeners are close to technical PCBs, which suggest chlorination of biphenyls as impurities of paraffins. The homologue profiles of PCBs are relatively lower chlorination pattern with some specific congeners.
These technical CPs are imported from China. The production of total CPs in China has continued to increase, reaching 600,000 tones in 2007. Although the huge production and use of CPs in China could imply potential contamination of various media, there is little information on exposure to SCCPs. In our previous study, the analytical methods for SCCP were investigated in detail and applied for dietary samples. Preliminary evidence on the significant increase of SCCP in food sample Beijing in 2009 warrants urgent investigations to refine dietary intake estimates by targeting food types and source identification.
On the other hand, the finding high levels of legacy POPs as impurities in technical CPs are more serious for POPs inventories around the world.

Novel aspects:
Identified high levels of POPs in technical CPs and products by GC-HRMS or GC-HR-Tof-MS

No.100887

# AQUEOUS-PHASE REACTIONS OF ATMOSPHERICALLY RELEVANT VOLATILE ORGANIC COMPOUNDS THROUGH TANDEM MASS SPECTROMETRY: AN INTRIGUING STORY OF AEROSOL FORMATION

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Keywords: volatile organic compounds, isoprenoids and their oxygenated metabolites, atmospheric aerosols, tandem mass spectrometry

Volatile organic compounds (VOC) are a broad group of species that play important roles in the environment. They come to existence in the air through different pathways, ranging from the vital activity of plant vegetation (a prevailing source) to anthropogenic emissions (a minor source). Regardless of the emission source, they are engaged in the formation of ambient secondary organic aerosols (SOA) in the atmosphere through a complex photo-oxidation chains followed by a gas-to-particle conversion. In addition, they affect human health, a quality of life, and in the larger scale the Earth's climate. A poorly understood chemical transformations of VOCs contribute to a significant fraction of organic matter in the atmosphere (~35% to ~90%) which is "frozen" in the form of aerosol particles.

Isoprene (2-methyl-buta-1,3-diene) and its first oxidation products (i.e., methacrolein, methyl vinyl ketone and methacrylic acid) are relevant volatile precursors of ambient SOA in the atmosphere. Isoprene is the most abundant non-methane hydrocarbon emitted to the atmosphere as a result of living vegetation. According to the recent data, the isoprene emission rate is estimated to be at the level of 700 TgC per year. While heterogeneous transformations of isoprene and its first oxidation products have been well documented, aqueous-phase reactions of these species with radical intermediates leading to the production of new class of wet SOA components such as polyols and their sulfate esters (organosulfates), are still poorly recognized. The chain reactions of isoprene with sulfoxy radical-anions (SRA) are one of the recently researched routes leading to the formation of organosulfates in the aqueous phase. The latter radical species originate from the auto-oxidation of $SO_2$ in the aqueous phase and are behind the phenomenon of atmospheric acid rain formation. This is a complicated chain reaction that is catalyzed by transition metal ions, such as manganese(II), iron(III) and propagated by sulfoxy radical anions.

The presented work addresses the chemical interaction of selected VOCs such as isoprene, methacrolein, methyl-vinyl ketone, with sulfoxy radical-anions in the aqueous solution under both dark and solar conditions. We showed that triple quadrupole mass spectrometry is a powerful technique to follow the chemical changes of monitored processes. The use of collision-induced dissociations revealed the formation of novel components of wet SOA, including oxygenated polar species with C-5 skeleton bearing $SO_3H$ (MW 182, 180) and $SO_2H$ (MW 166, 164) moieties on the hydroxyl group. The structures of these products were firmly confirmed by comparison of their liquid chromatography and mass spectrometry behaviors with that corresponding to the synthesized model compounds. It is believed that newly discovered highly polar low molecular weight compounds may contribute to the growth of wet aerosol particles by the formation of higher molecular weight species.

Novel aspects:
The presented study falls into a hot topic research on the formation, transformation and environmental implications of ambient aerosols (SOA). Our research revealed a number of novel wet aerosol components.

No.100895

# Rapid MS-profile of Bordô grape skin by MALDI-TOF-MS using different matrices

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Keywords: Bordô grape, MALDI-TOF, binary matrix,anthocyanins

Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry(MALDI-TOF-MS) has been applied for qualitative profiling of anthocyanins in Bordô skin grape extract. For the first time anthocyanis profile was analyzed by mass spectrometry in this type of local Brasilian grape. This work successfully demonstrates the capability of MALDI-MS to analyze anthocyanis using a mixture of matrices. For this purpose, three different MALDI matrices have been tested, such as α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (2,5-DHB) and the mixture of both. Anthocyanins are polyphenols responsible for the color and flavor of the grape and they are located mainly in the skin. They have antioxidant properties that may reduce the risk of cancer and heart disease. The most common anthocyanins found in grapes are delphinidin, cyanidin, peonidin, petunidin and malvidin. These pigments are present in their glicosylated form, where one or more of the hydroxyl groups are linked to sugars, mainly glucose and rhamnose. These compounds can bind phenolic acids, such as: coumaric acid, caffeic acid, vanilic and phenyl acid. The skin was carefully removed from the grape and the skin's compounds extracted with methanol. Just one microliter of the extract was spotted on the MALDI plate, after drying one microliter of matrix was added. The matrices were previously made up. Thirty mg of 2,5-dihydroxybenzoic acid was dissolved in 1 mL of MeOH. Ten mg of α-cyano-4-hydroxycinnamicacid were dissolved in a solution of acetonitrile and water ( 1:1) plus 2.5 % of trifluoracetic acid. A binary matrix solution was prepared by dissolving 7 mg each of 2,5-dihydroxybenzoic acid(DHB) and α-cyano-4- hydroxycinnamic acid (CHCA) in 1 ml of 70% methanol plus 0.1% TFA and 1% piperidine.The mass spectra were acquired in the 100-900 m/z mass range with a Autoflex III Smartbeam ( Bruker Daltonics) MALDI-TOF in reflector mode. The three matrices were evaluated. The spectra show the presence of 3-glucoside-p-coumarate (*m/z*801,09), petunidin 3-glucoside-coumarate-5-glucoside ( *m/z* 639,17), petunidin 3-glucoside-coumarate (*m/z* 615,15), peonidin 3,5-diglucoside (*m/z* 625,18) and malvidin 3-glucoside (*m/z* 331,05) with all the matrices. All the compounds observed were successfully confirmed by LIFT MS. Almost no matrix signals were observed in the lowest mass range with the binary mixture. Preliminary tests demonstrated the advantages to apply the binary matrix for MALDI-TOF-MS identification of anthocyanins in Bordô skin grape.

Novel aspects:
For the first time the anthocynins profile of Bordô skin grape was evaluated and a new selective method for their mass spectrometric analysis is proposed.

No.100902

# Determination of absolute configuration of PCB atropisomer and analysis of enantiomeric excess in the human sample

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Keywords: PCB atropisomer, human sample, circular dichroism, RI-CC2/TZVPP calculations, enantiomeric excess

The electronic circular dichroism(CD) spectra of a pair of enantiomeric 2,2',3,4,4',5',6-heptachlorobiphenyls (PCB-183) were investigated for the first time experimentally and theoretically. Geometrical optimization at the DFT-D3-B-LYP/TZVP level revealed that the two phenyl planes of PCB-183 are nearly orthogonal (89 ° ). Due to the sterically large chlorine atoms, PCB-183 becomes atropisomer. Thus, optical resolution was performed by chiral HPLC (column: OJ-H), affording enantiomerically pure first elute (100% ee) and optically enriched second elute (74% ee). Their experimental UV-vis and CD spectra in n-hexane were compared with those obtained by theoretical calculations at the RI-CC2/aug-TZVPP level.

Determination of absolute configuration is crucial in the structure elucidation and their biological activities of chiral compounds, and it is also true for PCB atropisomer. However, the determination of absolute configuration of PCB has never been performed. Here absolute configuration of PCB-183 (aS and aR) were determined, aS-PCB-183 and aR-PCB-183 in human sample are determined enantio-selectively.

Sample collection: The serum and adipose tissue samples were obtained after receipt of written informed consent. After about 10ml of blood were centrifuged by 3000 rpm, serum samples were obtained and stored in -20 ° C until analysis.

Extraction and cleanup: About 2g of serum was added cleanup spike solution, 2.5ml of diethyl ether, and 5ml of ethanol, then extracted with 10ml of hexane twice. Hexane extracts were passed through 1g of florisil/1g of silica gel double layer column (Supelco Inc., U.S.), eluted with 15ml of 15% diethyl ether/hexane. Eluate was concentrated to 0.1ml under gentle stream of nitrogen, and then added syringe spike solution[1)].

GC and LC conditions for chiral separation

To determine absolute configuration of each PCB atropisomers, the enantioselective separation of PCB atropisomer was performed using HPLC(TOSOH CO-8020, SHIMADZU LC-10AT) with CHIRALCEL (DAICEL) OJ-H (4.6mmIDx150mm). The adsorbent is cellulose tris(4-methylbenzoate) silica gel coatingtype, particle size 5 μm, sample loop : 20 μL, column temp : 38 ° C, n-hexane was used as elution solvent with flow rate 0.3mL/min, UV 291nm.

PCB-183 in human sample was enantio-selectively determined by GC/HRMS.(JEOL JMS-800D) using BGB-172 column (20% *tert*-butyldimethylsilylated *beta*-cyclodextrinin methylphenylcyanopropylpolysiloxane, 30m length x 0.25mmID, 0.25 μm Film Thickness, BGB Analytik AG). Carrier gas was helium,and injector and transfer line temperature were 230 ° C and 245 ° C. 1 μl of samples were injected splitless at an initial temperature of 120 ° C, 4 ° C/min to 180 ° C, 1 ° C/min to 230 ° C, and held for 10minutes. The ion source was operated in the electron-impact mode (EI, 38eV, 250 ° C).

Experimental data and theoretical calculation results

The concentrations is calculated as epsilon(209)=73000. Experimental CD spectra of aR/M are roughly similar in comparison with theoretical calculation of aS/P.(aS: axial S). In experimental CD spectra, comparison between fraction A(100%ee) and fraction B(74%ee) multiply 1.35 with inversion of sign were in excellent agreement. The first elution peak on BGB-172 column by GC/MS is assigned as aS/P. The first elution peak on OJ-H columnby LC/MS was also assigned as aS/P.

1)Chisato Matsumura et al, *Organohalogen compounds*, 69, 275-278, 2007

Novel aspects:

Absolute configuration of PCB-183 (aS and aR) were determined, aS-PCB-183 and aR-PCB-183 in serum are determined enantio-selectively.

No.100924

# Development and validation of methodology using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for monitoring for use of anabolic steroids in animals

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Keywords: anabolic steroids, urine, serum, LC-MS/MS

A wide range of anabolic steroids has been used in animal fattening because of their capacity to increase weight gain and the improvements in feed conversion efficiency. The use of anabolic steroids in animal fattening is prohibited in the European Community and monitoring for use of anabolic steroids is carried out through the National Plans of the individual Member States. For controls at retail level and for products imported in the EU, it is necessary to have analytical methods applicable to meat samples, whereas at farms and abattoirs,misuse of anabolic steroids in living animals is monitored by analyses of the animal's urine and/or serum. Because of the complexity of these matrices and the low "Recommended Concentrations" established, it is necessary to have sensitive, selective and specific methods for the detection of the anabolic steroids.

Methodology has been developed for the determination of 15 anabolic steroids in bovine, ovine and porcine urine. The procedure involved enzymatic hydrolysis prior to extraction and SPE prior to analysis by ultra high performance liquid chromatography coupled to a tandem mass spectrometer operating in electrospray, switching between positive and negative ion modes. Data acquisition was performed in selected reaction monitoring (SRM) mode. The method was successfully validated according to the Commission Decision 2002/657/EC for the detection and confirmation of residues in products of animal origin. The methodology developed is suitable for the detection, quantification and confirmation of identity of these anabolic steroids in bovine, ovine and porcine urine and can be used for residue control programs.

Novel aspects:
High sensitivity, validated methodology suitable for official control purposes, chromatographic and mass spectrometric selectivity, overcoming matrix effects

No.100929

# HRAM Screening and Quantitative Analysis of Pesticides in Environmental & Food Matrices using a bench top LCMS Orbitrap system

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Keywords: Pesticides, Orbitrap, LCMS, UHPLC

**Introduction:**
Current methodologies for the quantitation of pesticides infood revolve around using triple quadrupole platforms and long run times. The method described here utilizes UHPLC- witha second generation Exactive™ mass spectrometer (MS) using high resolutionaccurate mass. The second generation Exactive MS is capable of resolving powersettings of up to 140,000 (FWHM) at m/z 200, providing the ability to resolvematrix from analyte in full MS mode even in most complex matrices. This work describesa method to do screening and quantitation of a 100 pesticides mixture togetherwith high level confirmation.

**Method:**
A Hypersil Gold aQ C18 50x2.1mm 1.9u column was utilizedwith a run time of less than 8mins with all analytes eluting within 5mins. Astandard curve containing 110 compounds was spiked in orange solution rangingfrom 50 pg/mL levels to 250 ng/mL levels was injected in triplicate andscreening of different food matrix (green bell pepper and hot peppers) sampleswas analyzed for targeted list of 100 compounds and also screened for possibleother unknown pesticides. The spectrometer was set to a resolving power of 70,000(FWHM) at m/z 200 in full MS mode to minimize matrix interference, and all-ion-fragmentation(AIF) spectra were collected to qualify. The data was then compared to a current MS/MSlibrary for confirmation, as well as calibration curves were generated for theindividual compounds.

**Preliminary Data:**
Calibration lines were generated for the compounds analyzedwith $r^2$ better than 0.9940, the limits of detection (LOD) variedfrom 50pg/mL to 500pg/ml based on the individual compounds. For confirmation ofeach compound the exact mass of the compound, its isotopes and as well as the AIFproduced were collected and compared to limit the amount of "false positives"in the results. One of the mainchallenges using a high resolution accurate mass system is data mining, butwith novel ExactFinder™ software the data processing becomesstraightforward. Using the novel second generation Exactive the workflow toscreen and confirm in a single run increases throughput for repeat runs.

Novel aspects:
Usage of a novel Second Generation Orbitrap platform to screen, quantify and confirm a large number of pesticides at the low ppb level.

No.100972

# Analysis of natural organic dyestuffs extracted from textiles.

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Keywords: masspec, dyestuffs, natural, historical, textiles

Study of the fading of natural dyes in (historical) textiles is rather complicated because of low concentration of investigated chromophores plus a big surplus of matrice elements (textile fibres and degradation products) and because of the lack of significant portions of the testing material.

Most suitable method is extraction/soft hydrolysis by mixture of methanol formic acid (95 : 5) followed by LC-MS analysis. Most of the previous papers were concentrated on the analysis of chromophores themselves (aglycones) while glycosylated chromophores were not the principal target. The main reason was that the extraction by mixture of 37% HCl/MeOH/H2O (2:1:1) would hydrolyse O-glycosylated chromophores. Therefore, it is not possible to study the concentration changes of O-glycosides along with their artificial aging.

The overall goal of this work is to devise efficient techniques for extracting dyes from textile samples without simultaneously decomposing some of the dye components. Moreover, this type of extraction is more suitable for MS-analyzers, as HCl is a corrosion agent and, at the same time, formic acid can be readily evaporized after the extraction.

Silk textiles were dyed by mordant dyes (anthraquinones) extracted from the madder roots and exposed to two types of artificial aging (thermal and photooxidative), before and after the aging experiment. Analyses of the samples were carried out by extraction, 30 minutes-sonification at 60 ° C followed by evaporation of the solvents in a $N_2$-stream. Separation of analytes was carried out by gradient reverse phase chromatography on C18-column. Mobile phases were
water and/or acetonitrile both with 0,1% of formic acid. ESI in negative mode and HRMS detection using ORBITRAP technology were used for the analysis. Monitoring analytes were rubyretric acid (alizarin primeveroside), alizarin glucoside, alizarin, lucidin primeveroside, lucidin glucoside, lucidin, galiosin (pseudopurpurin proveroside), rubiadin primeveroside, rubiadin, purpurin.

Conclusions

During the first phase of colour fading of the analysed textiles the concentration of the aglycone itself (not bonded to the sugar moiety) is increasing because of the O-glycosidic bond breakdown (which is the most vulnerable to a fission). The moment all O-glycosidic bonds are completely hydrolysed, aglycone concentrations begin to sink. O-primeverosides first change onto O-glykosides of aglycones. The fotooxidation is more destructive then thermal conditions.

The most stable are O-glycosides and O-primeverosides of alizarin, which at the same time are the most concentrated chromophores in madder. Lucidin, rubiadin and pseudopurpurin are more degraded than alizarin. These facts lead to search first in historical textile for aglycons of chromophores, these are more stable.

The single MS spectra of glycosylated aglycones are always accompanied by typical daugther ions of the aglycones

$[M-162,053]^-$ for O-glucosides and

$[M-294,095]^-$ for O-primeverosides occuring in the ESI ionizator.

Novel aspects:

During the first phase of colour fading of textiles the aglycone concentration is increasing because of the O-glycosidic bond breakdown.The moment O-glycosidic bonds are completely hydrolysed, aglycone concentrations begin sinking.

No.100064

# Quantitative analysis of nine N-nitrosamines in human urine by isotope-dilution liquid chromatography- tandem mass spectrometry with on-line solid-phase extraction

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Keywords: N-nitrosamines, urine, on-line SPE, LC-MS/MS

*N*-nitrosamines are potentially carcinogenic, mutagenic and teratogenic compounds for animals and humans that are often found in food, drinking water and environment. In addition to exogenous exposure, *N*-nitrosamines can also be formed endogenously inside the body from its precursors, nitrate or nitrite and secondary or tertiary amines. In this study, we developed an isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) with on-line solid-phase extraction (on-line SPE) for a quantitative analysis of nine *N*-nitrosamines in human urine, namely *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosopyrrolidine (NPyr), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopiperidine (NPip), *N*-nitrosomorpholine (NMor), *N*-nitrosodi-n-propylamine (NDPA), *N*-nitrosodi-n-buthylamine (NDBA) and *N*-nitrosodiphenylamine (NDPhA).

A 24 ml of crude urine added with isotopic internal standards, then purified by activated carbon and concentrated to 0.1 ml under a high purity nitrogen stream. A 10 μl aliquot of prepared sample was then directly injected into the on-line SPE LC-MS/MS. Automatic sample cleanup (on-line SPE) was obtained by using a switching value (two-position microelectric actuator; Valco) and a C18 trap column (75 × 2.1 mm i.d., 5 μm, ODS-3, Inertsil) that was controlled using PE-SCIEX control software (Analyst; Applied Biosystems). After automatic sample cleanup, the sample was automatically transferred onto a C18 column (150 × 2.1 mm i.d., 5 μm, ODS-3, Inertsil) using a Agilent 1100 series HPLC system interfaced with a PE Sciex API 3000 triple quadrupole mass spectrometer with electrospray ion source (ESI) or atmospheric pressure chemical ionization (APCI). For a satisfactory sensitivity or selectivity, the quantifications of NDMA, NMEA, NPyr and NMor were performed in the positive-ion APCI mode, while the quantifications of NDEA, NPip, NDPA, NDBA and NDPhA were performed in the positive-ion ESI mode. For all of the samples, the $[M+H]^+$ ion was selected by the first mass filter. After collisional activation, two fragment ions were selected: the most abundant fragment ion was used for quantification (quantifier ion), and the second most abundant ion was used for qualification (qualifier ion). Optimal multiple reaction monitoring conditions were obtained for 36 channels: NDMA (m/z 75→43 and 75→58), NDMA-$d_6$ (m/z 81→46); NMEA (m/z 89→61 and 89→43), NMEA-$d_3$ (m/z 92→64); NPyr (m/z 101→55 and 101→41), NPyr-$d_8$ (m/z 109→62); NDEA (m/z 103→75 and 103→47), NDEA-$d_{10}$ (m/z 113→81); NPip (m/z 115→69 and 115→41), NPip-$d_{10}$ (m/z 125→78); NMor (m/z 117→87 and 117→73), NMor-$d_8$ (m/z 125→95); NDPA (m/z 131→89 and 131→43), NDPA-$d_{14}$ (m/z 145→97); NDBA (m/z 159→103 and 159→57); NDPhA (m/z 199→169 and 199→66), NDPhA-$d_6$ (m/z 205→175). The dwell times per channel were set at 50 ms for all the analytes and internal standards.

With the use of isotopic internal standards and on-line SPE, the detection limits of NDMA, NMEA, NPyr, NMor were 23.9, 6.2, 15.5, and 0.57 pg in APCI mode, while NDEA, NPip, NDPA, NDBA and NDPhA were 9.3, 1.7, 1.0, 0.34 and 0.14 pg in ESI mode, respectively. This method was further applied to measure the urinary concentrations of nine *N*-nitrosamines in nonsmokers. The results showed that among nine *N*-nitrosamines only NDMA (~0.34 ng/ml), NPyr (~1.35 ng/ml), NDBA (~0.015 ng/ml) and NDPhA (0.025 ng/ml) were detectable while the others were under LODs of our method. Overall, the present method would be useful to assess the human exposure to nitrosamines.

Novel aspects:
Quantitative analysis of nine N-nitrosamines in human urine by isotope-dilution liquid chromatography- tandem mass spectrometry with on-line solid-phase extraction

No.100074

# Hydrophilic-interaction liquid chromatography (HILIC)-tandem mass spectrometry for quantification of oseltamivir and zanamivir in surface water and sediment samples

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Keywords: Influenza, oseltamivir, zanamivir, river-water, sediment

Oseltamivir and zanamivir are the most prescribed antiviral drugs against influenza in Japan. Oseltamivir and its metabolite have been frequently detected in surface water bodies in Japan during the last few years particularly in seasonal influenza period posing the threat of emergence of drug-resistant genes in human pathogens. Although zanamivir is placed in the third position in terms of its use as an antiviral drug in Japan, its occurrence and fate in environmental waters are not known until now. This situation may be partially attributed to the lack of a suitable sample treatment and analysis methods for the drug. Therefore, suitable and sensitive sample pretreatment and analysis methods are highly desirable for quantitation of the drug in environmental waters. Furthermore, optimized pretreatment and analysis methods for both oseltamivir and zanamivir would be the best choice for their precise and rapid quantitation in water samples.

This article discusses the occurrence of oseltamivir phosphate (OP), oseltamivir carboxylate (OC) and zanamivir hydrate (ZH) in water and sediment samples from Neya River in Osaka using a recently developed novel solid-phase extraction (SPE) method for simultaneous recovery of the compounds and hydrophilic-interaction liquid chromatography (HILIC) -tandem mass spectrometry (MS/MS) method for quantitation. Water and top sediment samples at three representative points (ST-1, ST-2 and ST-3) from the top towards the mouth along Neya River were taken during the 2011/2012 seasonal influenza period. The ST-1 is characterized by very small river flow and relatively bigger size sand particles in sediment. The ST-2 is characterized by drastically large river flow due to wastewater treatment plant discharge in the upstream side and medium sand particles in sediment. The ST-3 is characterized by very large river flow and silt particles in sediment.

The OP, OC and ZH were not found in both water and sediment at ST-1. They were present in water, but not in sediment at ST-2. The scenario was completely different for ST-3. The three compounds were found in water samples, while OP only was present in the sediment at ST-3. Thus, the compounds were found in abundance in water phase, while OP alone was detected in the sediment from Neya River. Batch adsorption test results for the compounds in ultrapure water with river sediment showed the highest adsorbed fraction (24%) for OP, while the values for OC and ZH were 3% and 1% respectively. Moreover, the fractions in water phase for OC and ZH were about 97% and 93% respectively. The value for OP was only 6%. These results clearly indicated the highest adsorption affinity of OP to sediment, while the affinities for OC and ZH were negligibly small. It may be evident from these results that OP is more likely to be adsorbed to sediments than OC and ZH.

A newly developed SPE method for simultaneous recovery of oseltamivir and zanamivir together with a hydrophilic-interaction liquid chromatography (HILIC) -tandem mass spectrometry method were employed for accurate quantitation of the drugs in river water and sediment samples. The results demonstrated abundance of OP and OC in river water during 2011/2012 seasonal influenza period, while OP only exhibited high potential for adsorption to sediment. It became evident that monitoring the drugs, particularly OP, in water phase alone is not enough in assessing the threat of emergence of drug-resistant genes in human pathogens.

Novel aspects:
Novel SPE and HILIC-MS/MS methods are developed for extraction and quantitation of oseltamivir and zanamivir simultaneously in environmental waters. Oseltamivir is detected in sediment samples for the first time.

No.100116

# Determination of chlorinated/brominated polycyclic aromatic hydrocarbons (Cl/BrPAHs) in flue gas and ash from waste incinerator

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Keywords: halogenated PAHs, organohalogen compound, by-products

Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) such as chlorobenz[*a*]anthracene (ClBaA) and chlorobenzo[*a*]pyrene (ClBaP) have received worldwide attention because of their environmental persistence and widespread distribution. Horii et al. have showed that several ClPAHs and BrPAHs elicit dioxin-like activity with potencies comparable to those of several mono-ortho polychlorinated biphenyl (PCB) congeners. Recent reports have showed the occurrence of ClPAHs and BrPAHs in flue gas and fly ash from municipal and industrial waste incinerators. However, little is known about congener profiles and distributions of ClPAHs and BrPAHs in flue gas, fly ash, and bottom ash from waste incinerators, due to the lack of individual analytical standards. Several tens of individual ClPAHs and BrPAHs were therefore synthesized in our laboratory. In this study, we measured individual concentrations of 26 ClPAHs and 15 BrPAHs in flue gas, fly ash, and bottom ash from 8 waste incinerators. In addition, gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS) was applied for the comprehensive analysis of ClPAHs and BrPAHs congeners including unidentified congeners. Results showed that monochloropyrene (1-ClPyr) and 6-ClBaP were the dominant compounds in flue gas samples. The profiles of halogenated PAHs were similar to the profiles reported previously for urban air. Concentrations of chlorinated phenanthren and pyrene in flue gas were significantly correlated with the corresponding parent PAH concentrations. Significant correlation between $\Sigma$ClPAH and $\Sigma$PAH concentrations suggests that direct chlorination of parent PAHs is the mechanism of formation of ClPAHs during incineration of wastes. Furthermore, highly chlorinated PAHs and brominated/chlorinated PAHs were found in samples by the comprehensive analysis using GC-HRTOF-MS.

Novel aspects:
Gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS) was applied for the comprehensive analysis of ClPAHs and BrPAHs congeners including unidentified congeners.

No.100143

# Electrospray mass spectrometric observation of the interaction between environmental pollutants and biologic compounds

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Keywords: electrospray ionization; interaction; zinc pyrithione

Abstract

Interactions between biologic compounds and environment pollutants often cause various toxicities, such as defective development. Therefore, it is very important to understand the interactions.

Electrospray ionization mass spectrometry (ESI-MS) is a very powerful method for the characterization and identification of interactions between polar species, because the technique is a very soft ionization method. The technique has been frequently used for the analysis of the interactions between biological materials and chemicals (H. Moriwaki, 2002) or metal ions (H. Moriwaki, 2003).

However, it is very difficult to research the interaction between proteins and substances by ESI-MS, because there are many complex binding sites and forms between proteins and substances. There are several examples where an investigation of a solution of a target substance with amino acids by ESI-MS was studied in order to initially serve as a simple model for complex interactions of the substance in proteins (C. L. Gatlin and F. Tureek, 2000).

In this presentation, solutions containing amino acids and environmental pollutants were measured by ESI-MS for the purpose of gaining information on the behavior of the pollutants with amino acids and peptides. Zinc pyrithione (ZnPT) and toxic metal ions were selected as targets of this study (H. Moriwaki et al., 2009).

Zinc pyrithione (ZnPT) is the zinc chelate of 2-pyridinethiol-1-oxide. This compound is widely used as a bactericide, fungicide and algicide in various products, such as antidandruff shampoos. A few studies have examined the toxicity of ZnPT on aquatic organisms, and it has been clarified that ZnPT can be potentially highly toxic (K. Goka, 1999). Therefore, it is important to understand the influence of the compound on wild life and the mechanisms of the toxicity of ZnPT in order to determine the appropriate use of ZnPT. The ZnPT complex ($[\text{ZnPT-ligand + Amino acid}]^+$), in which the ligand of ZnPT was exchanged by the amino acid, was detected in ZnPT solutions mixed with one of 20 amino acids by ESI-MS. Histidine and cysteine, in particular, showed a high reactivity with ZnPT, while serine and glycine showed a low reactivity.

In addition, electrospray mass spectrometric observation of the interaction of toxic metal ions with amino acids has been also studied in the same manner as ZnPT.

The procedure described in this study is very simple and suggested only a simple model for the reaction of biological materials with the pollutants. However, it provides significant information for estimating the behavior of the pollutants within the living body.

Novel aspects:

Solutions containing amino acids and environmental pollutants were measured by ESI-MS for the purpose of gaining information on the behavior of the pollutants with amino acids and peptides.

No.100159

# Characterization of organic pollutants in River Water by GC/MS ion profiles

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Keywords: River pollutants, ion profiles, GC/MS

**Background.** The specific human activities lead to contamination of the aquatic environment with a wide variety of natural and synthetic compounds not found prior to modern times. Due to an incomplete elimination in wastewater treatment plant (WWTP) residue of contaminants are found both in waste and surface waters. Many of these compounds provide a means of identification sources of inputs and pathways of movement of chemicals through ecosystem.
In respect to this, one of priority tasks is to determine the environmental distribution of organic pollutants in the surface waters. Both anthropogenic and naturally occurring compounds are found mixed together in recent environmental samples and several of these compounds may be used as tracers to study natural processes affecting the fate and effects of chemical contaminants in water.

**Objective.** In the south-eastern of Europe there is little information on river water status[1]. The existent data refers at some metal trace in very critical sites, general quality studies by analyzing the benthic macrofauna but information on anthropogenic organic pollutants as individual compounds is limited to only few emerging contaminants[2]. Therefore a systematic structural investigation of individual molecular pollutant present in environmental water at regional level is crucial.
The present paper purpose is the Mass Spectrometric (MS) characterization of organic pollutants to evaluate the degree of contamination of the river waters in high populated catchments from South-Eastern of Europe. The samples were collected from few places along of the Prut River, Border River between Romania and Republic of Moldova. In the Prut Basin live more than 1000 000 inhabitants.

**Methods.** The samples were collected by three techniques: a) grab sample, b) using composite sample devices and c) by passive samples devices. The GC/MS analyses were performed using a Thermo Electron Polaris Q mass spectrometer operated in EI mode to 70 eV. The gas chromatograph was equipped with a capillary column HP-5MS (30x0.25mm) with 0.25film thickness. The temperature was programmed from $90^0$C to $315^0$C.

**Results.** The families of pollutants was visualised by chromatograms on diagnostic ions (base ion or ion of high intensity and molecular ion)[3]. A systematic study of individual compound as structure and quantity in region of border Romania-Moldavia was done based on experimental data on a big number of samples. Also was established the environmental molecular markers as identification of source pollution. The molecular marker compounds will be used in the subsequent monitoring activities to obtain the complete image of the specific pollutants in the Prut Basin in different seasons. An number of 67 of compounds were identified. The quantity of pollutants was calculated using isotopic labeled compounds. The structure and quantity of detected compounds are discussed in relation to sampling methods and collection places. The obtained profiles of pollutants are a function of their sources and reflect the industrial and domestic activities at regional level.

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Novel aspects:
The families of compounds are shown by ion chromatograms obtained on characteristic ions. Their origin is discussed in correlation with the compounds known as molecular markers.

No.100193

# Mass spectrometric analysis of 1,3,5-Trinitroperhydro-1,3,5-triazine (RDX)

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Keywords: RDX, Ion trap, ESI-MS, Explosive

1,3,5-Trinitroperhydro-1,3,5-triazine (RDX) is one of the most widely used explosives. In this study, RDX was directly infused into an electrospray ionization mass spectrometry (ESI-MS) system in negative ion mode. The RDX stock sample was prepared in a concentration of 1 mg/mL in acetonitrile (ACN). Then, the RDX stock sample was diluted in ACN (100-fold dilution) prior to the analysis. Direct infusion of the diluted RDX sample provided an adduct ion of RDX + $HCOO^-$ where HCOOH is believed to be originated from the decomposition of RDX. In positive ion mode, no RDX signal was observed in the current direct infusion ESI-MS analysis. The RDX samples spiked with acetic acid or benzoic acid were also analyzed, which provide an adduct ion of RDX + $C_2H_4O_2^-$ (m/z 280.9), RDX + $C_7H_6CO_2^-$ (m/z 343.1). Analysis of RDX spiked with ammonium acetate also generated an adduct ion of RDX + $C_2H_4O_2^-$ (m/z 281.1). The detailed experimental procedures and results will be provided during the presentation.

Novel aspects:
Mass spectrometric analysis of RDX using ESI-MS to investigate adduct ions of RDX, such as RDX + formate, RDX + acetate, and RDX + benzoate

No.100224

# Metal speciations in environmental and clinical applications

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Keywords: elemental-speciations, LC-ICP-MS, IIP, SPME, DRC

In the vast subject of trace analysis, evolutionary instrumentation and material developments are the current trends to re-invent the definitions of sensitivity and selectivity in the field of elemental speciations, which in fact has become an indispensible tool for environmental management and clinical monitoring. In the world's raising knowledge and demands for the quality of human health, the strategies of method development is conforming to the stringent requirements of analytical standards. A diversity of research work in this presentation aims to facilitate convenient execution of speciation protocols in routine monitoring processes. The novelty is exhibited at four levels, featuring high-throughput instrumental methods, simplified extraction with commercial tools, modified material for specialized applications and modulation of selective material with ion-imprinting technique.

In the first of our presented work, seven species of these elements, namely Cr(III), Cr(VI), As(III), As(V), monomethylated As, Se(IV) and Se(VI), were simultaneously determined by liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) with dynamic reaction cell (DRC) that offers optimized sensitivity at sub-ppb levels to the selected elements. The high through-put of this method has fulfilled the hustle routine of environmental water analysis [1].

Aiming at clinical monitoring application, the second part demonstrates a method of solid-phase microextraction-liquid chromatography (SPME-LC) developed for MeHg and EtHg speciation in complex biological matrix [2]. Organomercury speciation in patients' urine has revealed source and pattern of Hg exposure which should allow rapid diagnostic whereby appropriate clinical treatment can be applied.

The third part presents a novel laboratory-made SPE column packed with tetrabutylammonium hydroxide-immobilized activated carbon (AC-TBAH) for Se(IV) and Se(VI) enrichment. The assigned anion-exchange functionality on the porous AC surface offered outstanding enrichment factors for ppt-level speciation with LC-ICP-DRC-MS [3]. Speciation analysis in natural water samples further validated the robustness of the material which hence represents a low-cost substitute for anion exchange resins for routine applications.

Material design has been proven very useful for modulating selectivity for trace element analysis via the synthesis of ion-imprinting polymer (IIP), such as one demonstrated in final part of the presentation. After a series of selectivity investigations, imidazole-based arsenic imprinted polymer was selected for selective extraction and analysis of trace As ions in environmental application [4]. Tunable selectivity through monomer selection revealed a promising advantage of imprinting technique for future speciation method development. With growing maturity of speciation research, routine implementation in environmental and biological areas should make progress towards a sustainable human well-being.

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Novel aspects:
high-throughput hyphenated instrumentation; SPME-LC for speciation, modified material for sample preconcentration and simultaneous speciation; selectivity modulation using ion-imprinting technique

No.100244

# Occurrence of 92 pharmaceuticals in river water in agricultural or urban areas

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Keywords: Pharmaceuticals, Sewered Area, Unsewered Area

In recent years, physiologically active substances (e.g., pharmaceuticals) detected in the water environment have become an emerging public concern. Human-use pharmaceuticals enter raw sewage via urine and feces or by improper disposal. These pharmaceuticals are discharged from private households and hospitals, and eventually reach wastewater treatment plants. If pharmaceuticals are only partially eliminated, residual quantities enter the water environment. However, little information is available about the occurrence of pharmaceuticals in river water. The objective of this study is to determine the occurrence of pharmaceuticals in river waters characterized by land use of their river basins, such as agricultural or urban areas.

We conducted sampling on six fine days at two stations of a river in an agricultural area (St.1 and St.2) and two stations at a river in an urban area (St.3 and St.4). The catchment areas of each sampling site are 4.6km$^2$ (St.1), 4.1km$^2$ (St.2), 3.9km$^2$ (St.3) and 5.4km$^2$ (St.4). About 90% of land utilization of the catchment area of St.1 and St.2 consists of rice field, dry field, artificial forest, and broadleaf forest. Land utilization of the catchment area of St.3 and St.4 consists of a residential area (38% and 36%), school zone, open space, dry field, native grassland and artificial forest (54% and 56%), respectively. The catchment area of St.1 and St.2 is an unsewered area, and St.3 and St.4 are partly sewered areas. The percent of sewered population of the catchment area of St.3 and St.4 is 74% and 31%, respectively. The populations of each sampling site are 1,062 persons, 407 persons, 25,71 persons and 28,171 persons for St.1, St.2, St.3 and St.4, respectively. The population densities of the river basins are 231 persons/km$^2$ of St.1, 99 persons/km$^2$ of St.2, 6,594 persons/km$^2$ of St.3 and 4,661 persons/km$^2$ of St.4.

The analytical method of pharmaceuticals for river water samples is as follows: first, a 200ml sample was filtered through a 0.7-$\mu$m pore size glass fiber filter. After filtration of the sample, solid phase extraction was performed using an Oasis HLB cartridge. Subsequently, the cartridge was eluted with methanol. After solvent removal, the residue was dissolved in acetonitrile/water solution, which was then analyzed by LC/MS/MS. The procedure demonstrated in this study is innovative in terms of simultaneous analysis of 92 substances.

We identified a total of 48 substances (e.g., anti-inflammatory drugs, anti-epileptic drugs, antihypertensives, antibiotics, etc.) in river waters in agricultural and urban areas. The observed average concentration of pharmaceuticals ranged from 0.6ng/L to 14ng/L, 0.2ng/L to 52ng/L, 0.2ng/L to 670ng/L and 0.4ng/L to 1,500ng/L for St.1, St.2, St.3 and St.4, respectively. The numbers of pharmaceuticals detected in surveyed rivers were different. We found 15, 25, 37 and 45 for St.1, St.2, St.3 and St.4, respectively. In agricultural areas (St.1 and St.2), the numbers are much less than those in urban areas (St.3 and St.4). And also, the concentration of detected pharmaceuticals in agricultural areas (St.1 and St.2) is lower than urban areas (St.3 and St.4) and more pharmaceuticals were detected in St.4 than in St.3, which might be caused by the insufficient and discharge of wastewater in the catchment area of St.4. The number of detected pharmaceuticals is large when the population density is large, which is assumed to be caused by the pharmaceuticals used in society. In agricultural areas, detected numbers of pharmaceuticals were much less than those in urban areas and the concentration of detected pharmaceuticals in agricultural areas was also lower than in urban areas.

Novel aspects:
The objective of this study is to determine the occurrence of pharmaceuticals in river waters characterized by land use of their river basins, such as agricultural or urban areas.

No.100247

# Screening of five mycotoxins by using immunoaffinity column and HPLC-orbitrapMS in processed foods

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Keywords: Mycotoxins, OrbitrapMS, Immunoaffinity column

## Introduction

The risk assessment of mycotoxins contamination of foods was important because it can cause harmful health effects (e.g. cancer) in the humans. But the determination of mycotoxins in processing foods (e.g. snack, instant noodle) was a difficult experiment due to residue of extremely small amounts and adverse matrix effects (e.g. fat and sugar). The object of this study was to development a reliable and fast analytical method using by immunoaffinity column and HPLC-OrbitrapMS system in processing foods.
-Five mycotoxins: Aflatoxin, DON, Zearalenone, Ochratoxin, Fumonisin

## Materials & Methods

-Sample preparation: The immunoaffinity column was applied to ensure efficient cleanup. The homogenized 10g sample was weighed. 50mL of PBS (1X, pH 7.4, 1st extraction) and 50mL of 70% MeOH in DW (2nd extraction) were added for extraction. Two aliquot of the extract were transferred into immunoaffinity column for cleanup. After drying down eluant under an nitrogen stream at 50℃, reconstitute with 1mL of 40% MeOH in DW containing 1mM ammonium acetate and 0.1% acetic acid.
-LC/MS/MS analysis: 10uL of preparation sample was injected onto a XBridge C18 150*2.1mm, 3.5uL analytical column. A gradient LC method used mobile phases water containing 1mM ammonium acetate and 0.1% acetic acid and methanol containing 1mM ammonium acetate and 0.1% acetic acid at a flow rate of 0.2mL/min. HPLC-OrbitrapMS with ESI(+,-) probe was used and scan type was full scan.

## Data & Results

- MS Condition: Aflatoxin B1(313.0707,+), Aflatoxin B2(315.0863,+), Aflatoxin G1(329.0656,+), Aflatoxin G2(331.0812,+), Fumonisin B1(722.3957,+), Fumonisin B2(706.4008,+), DON(355.1387,+), Zearalenone(317.1394,-), Ochratoxin(404.0901,+)
- Linearity: The calibration curves had R^2 values that were greater than 0.98.
- Recovery: Each test was performed three times and two spiked level. In snacks, the mean recovery values range from 79.0% to 116.7% and in instant noodles from 73.8% to 94.7%.
- Repeatability: The RSD was calculated from three replicates. In snacks, the RSD range from 10.6% to 17.9% and in instant noodles from 8.5% to 14.4%.
- LOQ: The level of LOQ was low than the MRL established KFDA (Korea Food and Drug Administration) for mycotoxins. LOQ range of aflatoxin, DON, zearalenone, ochratoxin, and fumonisin were 0.1, 10.0, 5.0, 0.5, and 10.0ug/kg respectively.

## Novel aspects:

Applying to processing foods, Using OrbitrapMS, Muti-determination of mycotoxins

No.100251

# Application of tandem mass spectrometry for the structure confirmation of a wide range of peptides synthesized by cyanobacteria Woronichinia naegeliana

Beata Bober[1,2], Zbigniew Lechowski[2], Jan Bialczyk[2], Piotr Suder[3], Ken-ichi Harada[1]

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Keywords: Cyanobacteria, peptides, MS/MS, structure confirmation

Introduction

The eutrophication of water caused by human activity has influenced the increasing of mass appearance of cyanobacteria. The cyanobacterial blooms are undesirable because of ecological as well as health reasons. Cyanobacteria have a remarkable ability to synthesize and release into the water a wide range of bioactive secondary metabolites. Most of them are oligopeptides, which in terms of molecular structure are divided into linear (aeruginosins, microginins), cyclic (anabaenopeptins, cyanopeptolins, microcystins, cyclamides) and multicyclic (microviridins). Some of them have been recognised to cause accute or chronic toxicity and might be reasons for serious health problems, others could be an attractive resources for new drug discoveries. The determination of these compounds is of great significance because the cyanobacteria that produce them often occur in reservoirs of water designated for consumption. *Woronichinia naegeliana* (Unger) Elenkin is a species of cyanobacteria belonging to *Chroococcales* and is appearing increasingly frequently in fresh water areas worldwide. Outbreaks, particularly in late summer and autumn, have been confirmed in Europe, North America and Australia. However, to date its secondary metabolites have not yet been determined adequately. As a result of the potentially significant consequences that can result from a bloom of *W. naegeliana*, research has been undertaken to identify its secondary metabolites. Taking into consideration limitations of applicated analytical methods such a high performance liquid chromatography (lack of standards) and nuclear magnetic resonance (a high amount of sample is required), only the using tandem mass spectrometry (MS/MS) with electrospray ionization allows to confirm structures of compounds synthesized by *W. naegeliana* with a high degree of specifity.

Method

Field samples of *Woronichinia naegeliana* were collected from blooms in Dobczyce Reservoir (southern Poland). The obtained extract from the lyophilized cyanobacteria cells was concentrated by solid phase extraction (SPE). Separation and preliminary analysis of the samples were carried out using a high performance liquid chromatography system with photodiode array detector (HPLC-PDA). The isolated fractions were analyzed with a mass spectrometer with an electrospray ion source (ESI-MS). The positive-ion mode was applied. The scan range was m/z 200-1400 in the MS and MS/MS modes. Because purified standards of the majority of metabolites produced by cyanobacteria are not available for identification, confirmation of the identity of a given compound was done by interpretation of the MS/MS spectra.

Results and Discussion

The obtained product ions were quite effective for the amino acid sequencing of linear as well as cyclic cyanopeptides. In the case of cyclic peptides, the characteristic fragment or series of fragment indicating the cleavages at definite peptide bonds were helpful for the interpretation of fragmentation pattern. On this base the peptides isolated from field samples of *W. naegeliana* fell into four classes: microginins (microginin 757, microginin 91E, microginin FR3, microginin FR4), cyanopeptolins (cyanopeptolin B, cyanopeptolin C, cyanopeptolin D, cyanopeptolin 880, micropeptin 478-B, micropeptin 88D), anabaenopeptins (oscillamide B) and microcystins (trace amounts of microcystin-LR). The molecular masses of the determined metabolites range from 700 to 1100 Da. The particular groups of structurally related compounds showed similarity of the fragmentations patterns. These results confirm the usefulness of MS/MS for the determination of linear and cyclic cyanopeptides with a high structural diversity.

Novel aspects:

The structure confirmation of a wide range of bioactive peptides synthesized by cyanobacteria Woronichinia naegeliana can be successfully performed using MS/MS.

No.100287

# A search for active ingredients in cigarette smoke that modify significant biomolecules

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Keywords: Cigarette smoke extract, L-tyrosine, LC/MS, LC/MS/MS, GC/MS

Cigarette smoke contains a number of toxic chemicals, many of which contribute to the pathogenesis of smoking-related diseases such as chronic obstructive pulmonary disease, vascular disease and cancer. Modification of biomolecules by the harmful chemicals present in cigarette smoke is thought to mediate the adverse health effects of smoking. The chemical analysis of such active ingredients in cigarette smoke is one of the most challenging tasks for analysts, and extensive work has been done using many analytical techniques. Despite such work, there have been no reports regarding the analysis of reaction products from the compounds incigarette smoke and functional biomolecules.

The purpose of this study is to search for biomolecules modified by active ingredients in cigarette smoke. We tried to identify the products formed by reaction of gas-phase cigarette smoke extract (CSE), from which nicotine and tar had been removed, with L-tyrosine (Tyr) on the assumption that Tyr is a key target aminoacid in proteins for cigarette smoke toxicity. A highly sensitive LC/MS/MS system was utilized for detection and identification of trace amounts of there action products. We also tried to identify and quantify the active ingredients in CSE by GC/MS or LC/MS.

CSE was prepared by bubbling into phosphate-buffered saline (PBS) (1 mL per three cigarettes) the main stream of smoke (gas phase) from which the particulate phase including tars and nicotine had been removed 99.998% by passage through a Cambridge filter. The pump flow rate was kept constant (1 L/min) and smoke was bubbled only for 1 min after lighting a cigarette. The resulting solution was designated the 100% CSE solution and stored at -80℃ until it was used. A Quattro Premier triple-quadrupole LC/MS (Micromass, Manchester,UK) with an ESI source was used for the positive and negative ion mode Q1 scan and MS/MS analysis coupled to the Alliance HT 2795 Separations Module (WatersCo., Milford, MA, U.S.A.). A mass spectrometer (Automass SUN, JEOL Ltd., Tokyo, Japan) equipped with a GC (6890N, Agilent Technology Inc., Santa Clara, CA, U.S.A.) was used for identify and quantify the active ingredients in CSE.

We found that CSE readily reacts with Tyr at body temperature (37℃) to form various Tyr derivatives, which can be detected using a LC/MS and LC/MS/MS. From among these derivatives, we could identify two acetylated compounds, *N*-acetyl-Tyr and *O*-acetyl-Tyr, and a new compound, *M*r251 (Tyr+70).

Next, to identify and quantify the compound of *M*r 251 (Tyr+70) in CSE we used GC/MS analysis. We found that two peaks indicate *M*r 70 from *m/z* 70 chromatogram of CSE. From the result of the library search of their mass spectra, we identified the peak for $t_R$ 9.5 min as crotonaldehyde and the peak for $t_R$ 6.3 min as methyl vinyl ketone, as candidate ingredients. These retention times were the same as those of their respective authentic samples. Thus, to clarify the structure of compound Tyr+70, the authentic crotonaldehyde and methyl vinyl ketone were directly reacted with Tyr at 37 ℃. The reaction products were analyzed by LC/MS in the SRM mode. The peak of compound Tyr+70 appeared on the SRM spectra of the mixed solution of Tyr and methyl vinyl ketone, but was hardly detected on that of the mixed solution of Tyr and crotonaldehyde. The analytical data of compound Tyr+70 synthesized by reaction of methyl vinyl ketone with Tyr completely agreed with those of compound Tyr+70 obtained by reaction of CSE with Tyr. This indicates that methyl vinyl ketone in CSE reacts with Tyr to produce compound Tyr+70. The structure of compound Tyr+70 was identified as *N*-(3-oxobutyl)-Tyr[3-(4'-hydroxyphenyl)-2-(3"-oxobutylamino)propanoic acid].

Novel aspects:
We found out that active ingredients in CSE readily react with Tyr to form various Tyr derivatives containing a new compound, suggesting that cigarette smoke can modify biogenic amino acids.

No.100292

# Untargeted screening of pesticides metabolites by LC-HRMS: a tool for human exposure evaluation?

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Keywords: Pesticides, screening, untargeted, HRMS, metabolomics

The estimation of human exposure to pesticides still represents a challenge since sample amounts available from a human cohort study are often very low, and the search for possible compounds has to be as thorough as possible. From urine samples, pesticides are generally detected as metabolites whereas all possible metabolites structures could be unknown. In this context, this work aimed at the assessment of an untargeted approach using UHPLC-HRMS to characterize pesticides metabolites in urine samples from the "PELAGIE" (endocrine disruptors: longitudinal study on pregnancy anomalies, infertility and childhood) human cohort study. The PELAGIE study was drawn to evaluate the consequences of the exposure to multiple contaminants, and particularly to determine the influence of the exposure to pesticides on pregnancy, birth and psychomotor growth of the child. This study was conducted on a representative cohort of 3421 pregnant women living in a French rural area (Brittany).
In our work, 40 samples were randomly selected from 4 groups of individuals variously exposed to pesticides on the basis of their environment: urban population versus rural population surrounded by more or less cereal cultures. These samples were directly analyzed by UHPLC-HRMS (stationary phase C18, Electrospray ionization in the positive and negative mode, LTQ-Orbitrap mass spectrometer). Obtained data were processed with the MetWorks software (Thermo Scientific) to extract and integrate HRMS signals of 47 pesticides and their known or theoretical metabolites. Moreover, a major advantage of this approach is the possible detection of compounds which are not present in the initial metabolites list. Up to now, almost 450 substances (pesticides + putative urinary metabolites) were monitored by this way. Following their detection by UHPLC-HRMS, $MS^n$ experiments were performed to confirm or not, the detected compounds as potential or probable metabolites. Some of them have also been confirmed by comparison with metabolites generated during a parallel animal experimentation.
From human samples, 24 metabolites were identified using ESI in the negative mode and integrated. Data obtained by ESI in the positive mode could only confirm the identification of the metabolites detected in the negative mode, but did not allow the characterization of new compounds. Data were then processed by PLS-DA after an OSC filtration. The best separation of samples groups was obtained by the data normalization with the use of an internal standard during UHPLC-HRMS experiments. According to the model generated by this way, the separation of individuals was explained by 6 variables representing 3 pesticides, among which 2 fungicides classically used in cereal cultures were found.
The semi-targeted method developed in this work allowed to distinguish various groups of individuals according to their exposure to pesticides, on the basis of several urinary metabolites. The acquisition of full scan HRMS signals allows having complete datasets available to setup targeted MS/MS experiments for the structural identification of the metabolites detected in a first attempt.

Novel aspects:
The semi-targeted method developed in this work allowed to distinguish various groups of individuals according to their exposure to pesticides, on the basis of several urinary metabolites.

No.100301

# Silicon speciation in petroleum products using a multi-technical approach by Mass Spectrometry for a better understanding of catalyst poisoning

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Keywords: silicon speciation, catalyst poisoining

Silicon speciation is of considerable importance in the oil and gas industry due to the negative effect of its relative species on the performance of hydrotreatment catalysts. In petroleum products, silicon mainly comes from the use of antifoaming such as polydimethylsiloxanes (PDMS) to avoid emulsions in the different processes such as cokefaction, visbreaking, steamcracking or distillation and to enhance the crude oil recovery from the reservoir. PDMS, consisting in a structural unit of $(CH_3)_2$-Si-O-, has excellent properties of low surface tension and initial great thermal stability. Due to the high temperature applied in thermal cracking processes, PDMS degrades around 300 ° C and mainly generates cyclic siloxanes ($D_n$) with also many different silicon species in petroleum products that can affect the performance of catalysts.

In the oil and gas industry, the possible reactivity of hydrocarbon radicals with PDMS degradation products, was never reported and the chemical nature of these related silicon species must be determined. Up to now, these issues have been well identified but only addressed through the total silicon determination. These results have displayed total concentration levels ranging from $\mu g.kg^{-1}$ of Si up to some $mg.kg^{-1}$ of Si in petroleum products measured by elemental techniques. Moreover, the complexity of petroleum matrices, the wide variety of compounds concerned combined with possible contamination problems occurring during silicon analysis has hampered the development of speciation studies.

A very innovative and complete multi-technical approach based on MS techniques was developed and applied to identify and quantify silicon species. PDMS degradation samples, produced under thermalcracking of hydrocarbons on a pilot plant and several naphtha and gasolines coming from different refining processes were investigated. For low molecular weight silicon compounds, gas chromatography (GC) hyphenated to MS in single ion monitoring (SIM) was performed in gasolines. Cyclic siloxanes were confirmed as the major degradation products with trace of linear siloxanes. However, suspected unknown silicon compounds cannot be detected as their structures and fragmentations patterns remained unknown. To overcome this challenge, FT-ICR/MS was performed in positive electrospray mode (ESI) and allowed the characterization by their raw formula of more than 50 new silicon compounds with several unsaturations in naphtha and gasoline samples. To obtain a quantification and a structural identification of these compounds, GC-ICP/MS providing a specific detection combined to GC-GC/MS addressing the chemical structure were carried out both in PDMS degradation samples and in naphtha and gasolines. Finally, MS/MS experiments were also carried out to confirm the suspected chemical structures.

More than one hundred silicon species were highlighted in this work. Silicon compounds present in PDMS degradation samples and in gasoline were compared and confirmed the correct representativity of the evaluated conditions of PDMS degradation samples. The presence of PDMS, intermediate polymers and low molecular weight silicon compounds could explain the distribution of silicon amount in the different cuts of petroleum products and probably the poisoning effect on hydrotreatment catalysts.

Novel aspects:

Due to the thermal degradation of PDMS in the presence of hydrocarbons, more than 100 silicon species were highlighted and could act as a poison on hydrotreatment catalysts

No.100327

# Development of smoke diagnostic assays: When the smoke clears, will it end up in the wine bottle?

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Keywords: volatile phenols, phenolic glycosides, HPLC-MS/MS, smoke, bushfires

Smoke from forest/bushfires across winemaking regions in Australia as well as overseas has caused much concern among grape-growers and winemakers. Wines made from smoke-affected grapes have been described as 'smoky', 'dirty', 'ashtray' and 'burnt'. To aid purchasing, harvesting and winemaking decisions following a smoke event, winemakers and grape-growers clearly need reliable diagnostic strategies to assess the impact of smoke exposure in grapes as early as possible, ideally before harvest.

Measurement of the smoke marker compounds guaiacol and methylguaiacol in grapes has been commonly carried out as the smoke diagnostic assay and can identify severely smoke-affected samples. However winemakers have reported increasing numbers of wine samples developing undesirable smoke-related characters during and after winemaking, even when grape guaiacol concentration was as low as natural abundance. Additional challenges are that guaiacol can be found in non-smoked grapes as a natural component, and is extracted from toasted oak, so interpretation of wine data is complicated. These observations strongly suggested that the better smoke marker compounds to assess the extent of smoke exposure on grapes and wine were needed.

To find new smoke marker compounds and develop practical and reliable smoke diagnostic assays, various mass spectrometric techniques were applied to the following experiments;
• analysis of smoke generated by a forest fire to find major smoke components
• uptake of the major smoke components by grapes
• metabolism of the major smoke components in grapes
• identification of metabolites of the major smoke components in grapes
• extraction of the metabolites from grapes to wine
• evaluation of the metabolites as smoke marker compounds
• development of methods for the quantification of the metabolites used as smoke diagnostic assays

As a result, various glycosidic forms of guaiacol, methylguaiacol, syringol, methylsyringol, *o*-, *p*- and *m*-cresol, and phenol were selected as smoke marker compounds. Advantages of the use of these glycosides were as follows;
• when a grapevine is exposed to smoke, the amount of the volatile phenols taken up by grapes can be related to the intensity and duration of smoke exposure
• once taken up by grapes, the volatile phenols are rapidly metabolized into their more stable and non-volatile glycosidic forms
• the smoke induced glycosides persist and accumulate in grapes until the time of harvest, therefore, the amount of the grape glycosides can be correlated to the intensity of smoke exposure, and possibly also used to quantify extended or repeat smoke exposure
• the phenolic glycosides are not present in non-smoked grapes and are also not found (unlike free guaiacol) in oak in significant concentrations;
• the phenolic glycosides are easily extracted into wine and act as a pool of precursors to release volatile phenols during alcoholic and/or MLF fermentation, aging, and storage. This implies that measurement of phenolic glycosides can be used to estimate the potential of grapes to produce smoke-related taints after fermentation and at the time of consumption.

Novel aspects:
The newly developed methods based on the analysis of grapes and wine for phenolic glycosides induced by smoke exposure were proven to be sensitive and reliable as smoke diagnostic assays

No.100346

# Concentration profiles of PCB congeners in Steller sea lion, Hokkaido, Japan

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Keywords: PCBs, congeners, fetus, liver, blubber

## Introduction

PCBs still have been the major contaminants in the marine mammals at the top of the marine food-web. Especially, liver and blubber were thought to be most accumulative organs of PCBs in their body. The high risks of PCBs on the immunity obstruction and the cacochymia in marine mammals are feared. Therefore, more detail survey about PCB congeners in marine mammals is necessary.

In this study, PCB congeners in the liver and blubber of Steller sea lion (*Eumetopias jubatus*: SSL) from the coastal Hokkaido, Japan were measured by HRGC-HRMS to describe the distribution of PCB congeners in the males, females and fetus of SSL.

## Materials and Method

The liver and bluber samples of SSL were obtained from commercial fishery-related control killed and bycatch animals at the Shakotan area and Nemuro Strait, Hokkaido, Japan, during winter season in 2008 and 2010. These samples were measured by an isotope dilution method with $^{13}C$-labeled internal standard substances. The liver samples of eight males, two females and three fetuses were analyzed. The blubber samples of four males and three females and three fetuses were analyzed. These samples include three pairs of mother and fetus.

## Results and Discussion

In the males, #153 (in IUPAC#) was predominated in the both organs of the liver and blubber, followed by #138, #99 and #118. The concentration levels of #28, #31, #177 and #199 were appeared to be considerable differences between these tow organs. It seemed that each organ had specific accumulative property of PCB congeners. The total PCB concentrations ($\Sigma$PCBs) of males in the liver and blubber were 3,100–1,300 ng/g-fat and 3,000–830 ng/g-fat, respectively.

Between genders, the concentration profiles of major congeners in the blubber were differed, significantly. Meanwhile in the liver, there were no obvious differences in the congener profile between the male and female. In general, gender differences depend on the experience of pregnancy and lactation. $\Sigma$PCBs of the female in the liver and blubber were 1,200–810 ng/g-fat and 910–410 ng/g-fat, respectively. $\Sigma$PCBs in female were 61%-71% less than in males.

We analyzed PCBs in the liver and blubber from three pairs of mother and fetus to describe the detail profiles and to compare the concentrations between them. In the blubber, #153 and #138 were dominated congeners except one fetus. The major congeners in the blubber and liver of fetus were #153, #138 and #118 except one fetus. The average of $\Sigma$PCBs in the liver and blubber of the fetus was 710–430 ng/g-fat and 1,600–900 ng/g-fat, respectively. It was explained that $\Sigma$PCBs in the blubber of fetus was about 27%-85% higher than those of mother. These fetuses already have been contaminated during gestational period. It was thought that the concentration levels of these fetuses might depend on each mother's PCB levels.

The concentration profiles of congeners in the liver and blubber were different between the liver and blubber of males, the blubber of genders, each pair of mother and fetus. The level of $\Sigma$PCBs in male SSL from Hokkaido was as the same level as the report in 14 years ago (Kim et al.,1996). The PCB levels of congeners in SSL from Hokkaido showed how PCBs still remained in marine environment of the coastal Hokkaido. More investigations of PCB congeners in SSL are needed to study the mechanism of the accumulation and metabolism.

Novel aspects:
The distributions of PCB congeners in SSL were described. The concentration profiles of PCB congeners in the liver and blubber were different among males, females and fetuses.

No.100367

# ATMOSPHERIC PRESSURE PHOTOIONIZATION-MASS SPECTROMETRY OF ESTROGENIC COMPOUNDS

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Keywords: APPI, UHPLC, Mass Spectrometry, Estrogenic Compounds,

Among endocrine-disrupting chemicals (EDCs), natural and synthetic estrogens are considered the most potent estrogenic compounds. EDCs are widely distributed over the aquatic environment and due to their ecotoxic effects natural and synthetic estrogens are of special relevance even at very low concentrations. Estrogenic compounds are usually excreted into the aquatic environment through human and animal urine and the use of estrogens in medicine or in veterinary have caused their presence in aquatic ecosystems [1]. Liquid chromatography coupled with tandem mass spectrometry using both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) is the technique of choice for the LC-MS analysis of these compounds [2,3]. However, estrogens are non polar compounds and are usually hard to ionize using classical LC-MS sources mainly ESI. Atmospheric pressure photoionization (APPI) which can ionize both polar and non polar molecules with high sensitivity can be considered an alternative and has began to be used for the analysis of these compounds [4]. An additional advantage of using APPI is that ionization can be enhanced adding dopant substances and in some cases the choice of an adequate dopant is as much important as the choice of the source of ionization itself [5].

The aim of this study was to compare the behavior ofthree ionization sources (ESI, APCI and APPI) on analyzing eight estrogenic compounds (dienestrol, diethylstilbestrol, estrone, 17 $\beta$-estradiol, 17 $\alpha$-estradiol, mestranol, 17 $\alpha$-ethinylestradiol and estriol). LC-MS was carried out using an Accela liquid chromatograph system coupled to a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (ThermoFisher Scientific). To evaluate APPI performance six dopant solvents (toluene, acetone, ethylacetate, anisole, clorobenzene, tetrahydrofuran) as well as some dopant mixtures (toluene-anisole and tolune-chlorobenzene) were used and their sensitivity and their capability to ionize estrogens was compared. Negative ESI mode only provided the deprotonated molecules as base peak and low responses while in positive APCI the dehydrated fragment ion was found for the majority of the estrogenic compounds and better signals were obtained. As regards APPI in general most of the dopants provided the positive radical ions as the most abundant peaks. However, when using anisole or the mixture toluene-anisole as dopants the protonated ion $[M+H]^+$ resulted favored against the positive radical ions. For all the estrogenic compounds the highest signal responses were found with chlorobenzene and the mixture toluene-chlorobenzene except for dienestrol that gave higher signals using anisole, although signal-to-noise ratio worsened due to dopant solvent ionization. The detectability of the selected estrogens by APPI using chlorobenzene was found to be more effective than that of APCI and ESI, and was then selected for the analysis of estrogenic compounds.

A fast and high-efficient chromatographic separation of all estrogenic compounds was achieved in less than 4 min with an Ascentis Express Phenyl-Hexyl HPLC column (150 mm x 2.1 mm i.d., 2.7 $\mu$m) and gradient elution using water and acetonitrile as mobile phases. Good method performance was observed in terms of LODs (ppt level), linearity and run-to-run precision (RSD < 4%). The method was applied to the analysis of wastewater samples from a Hospital after off-line SPE using HLB cartridges, and estrone and estriol were detected and quantified at low ppb levels.

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Novel aspects:
Study of the effect of different dopants in the APPI ionization of estrogenic compounds

No.100410

# Photo ionisation time-of-flight mass spectrometry as a powerful tool for the on-line analysis of tobacco and wood combustion and pyrolysis

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Keywords: Photo ionisation time-of-flight mass spectrometry, biomass, combustion, pyrolysis, PAH

First, a coupling between a cigarette smoking simulator and a time-of-flight mass spectrometer was built to enable the sampling and analysis of fresh tobacco smoke under simulated burning conditions which are closely related to the conditions of a real burning cigarette. However, the cigarette smoking simulator also allows cigarettes to be "smoked" apart from normal burning conditions by a flexible control of parameters such as smouldering and puff temperatures as well as combustion rate and puffing volume in order to allow an investigation of the compounds' formation mechanisms and pathways. The first study of the system included the "smoking" of reference cigarettes (here: 3R4F) under nitrogen atmosphere to separate pyrolysis from combustion processes.
The second part addresses a measurement campaign, which was carried out at the Technology and Support Centre (TFZ, Straubing, Germany), concerning wood combustion in a normal stove under several normal and malfunctioning conditions, which includes the usage of spruce and beech wood as well as the usage of artificially dried and wet wood. The sampling point of the mass spectrometer was located in the raw exhaust pipe. The objective of the study was the correlation of certain burning phases with the formation of several health relevant compounds such as polycyclic aromatic hydrocarbons (PAH).
Both approaches enable the direct sampling and analysis of almost unaged smoke which is a complex and dynamic matrix. Therefore, time-of-flight mass spectrometry together with photo ionisation (SPI = single photon ionisation; REMPI = resonance enhanced multiphoton ionisation) was applied to analyse these mixtures on-line with a high time resolution. Both photo ionisation techniques are unable to ionise prominent bulk compounds of combustion emissions such as nitrogen, carbon dioxide or water. In addition, REMPI is highly selective and sensitive for the detection of phenols and PAH.
The two systems demonstrate clear distinctions between the different experimental conditions based on their corresponding mass spectra and further statistical evaluations such as principal component analysis. In the first experimental setup the yield of nearly all compounds decreased while changing the burning atmosphere from inert to oxidative. Other compounds such as benzene and phenol were not significantly influenced by the type of burning atmosphere. The second setup reveals that the maximum compounds' yields can be measured over a few minutes directly after each new load of wood and during the experiments involving the artificially dried and the wet fuel. Partially, a prompt change from pyrolysis to combustion could be observed by monitoring their particular marker compounds.

Novel aspects:
The first hyphenation of a PI-TOFMS and a cigarette smoking simulator as well as a stove was utilised to reveal formation mechanisms and pathways of health relevant compounds during combustion.

No.100423

# Determination of Volatile Organic Compounds in Polypropylene Raw Materials by Thermal Desorption-Gas Chromatography/Mass Spectrometry and Head Space-Gas Chromatography-Mass/Spectrometry

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Keywords: VOCs, HS-GC/MS, TD-GC/MS

Thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) and head space-gas chromatography-mass/spectrometry (HS-GC/MS) had been applied to the determination of volatile compounds emitted from polypropylene (PP) raw materials.

During the TD analysis, 0.30g of sample was heated in a stainless steel tube at 90℃ for 3 min, the emitted volatile substances was cooled with the help of a helium gas stream in the cold trap. After completion of the baking phase, those absorbed in the cold trap are rapidly heated from -10℃ to 280℃. The target compounds were evaporated and then isolated in the gas chromatographic separating column and detected by the mass spectrometer. Chromatographic separation was carried out on an HP-5MS column (30m×0.25μm×0.25mm) with a temperature program, and the mass spectrometric detection was operated by a quadrupole mass spectrometer with an electron impact ion source. The mass spectrums of each chromatograph peak obtained from the TD-GC/MS method were searched in the NIST Mass Spectral Library to obtain the qualitative results.

And the HS technique had been carried out at 120℃ for 5 hours, 4.50g PP raw materials were sealed in a 20mL HS glass bottle which was heated in the head space sampler. The head space gas which composed of air and the volatile matter were injected by the headspace sampler into the inlet of the gas chromatography. The GC/MS experimental conditions were the same as described above.

A series of PP raw materials were detected by both of HS-GC/MS and TD-GC/MS analysis methods. The research results showed that the volatile organic compounds (VOCs) released from the PP raw materials were composed of alkanes, alkenes and oxygen compounds with low molecular mass. Compared with HS method, the results of TD technology attained a large number of chromatographic peaks from which indicated that more substances would be determinate and showing a wide application prospect in the determination of the VOCs of the PP raw materials.

Novel aspects:

The TD-GC/MS methods was used in the determination of volatile compounds emitted from polypropylene (PP) raw materials, and campaired with HS-GC/MS method.

No.100506

# High Resolution LC-MS for Screening and Quantitative Analysis of Antibiotics in Drinking Water using an Orbitrap and Online Sample Preparation

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Keywords: Antibiotics, Orbitrap, Online SPE, PPCPs

Most current methodologies for the quantitation of antibiotics in drinking water revolve around analysis using triple quadrupole platforms with offline sample preparation. The method described here utilizes LC-MS(/MS) with a Orbitrap instrument using high resolution accurate mass and online sample preparation. This work will describe a method to do screening and quantitation of 27 antibiotics at ppb and sub ppb levels in drinking water using online pre-concentration together with high resolution accurate mass confirmations of the compounds.

A standard curve containing 27 compounds was spiked in neat solution ranging from 100 pg/mL levels to 250 ng/mL levels was injected in triplicate and screening of different water samples (municipal tap water and bottled water) was analyzed for a targeted list of the 27 compounds. The sample was also screened for other possible unknown pesticides compounds. The spectrometer was set to a resolving power of 70,000 (FWHM) at m/z 200 in full MS mode to minimize matrix interferences, and data dependant all-ion-fragmentation (AIF) was collected to quantify and qualify. The data was then compared to a current MS/MS library for confirmation, and calibration curves were generated for the individual target compounds.

Calibration lines were generated for the compounds analyzed, and the limits of detection (LOD) varied from 100pg/mL to 500pg/ml based on theindividual compounds based on a 1 mL injection. For confirmation of each compound, the exact mass of the compound, its isotope pattern and as well as the MS/MS spectrum produced were collected and compared against a fragmentation library which prevented "false positives" in the results. One of the main challenges using a high resolution accurate mass system is data mining. In this work we will show the data to screen, quantify and confirm in a single run therefore, spending less time on the instrument for repeat runs. Furthermore, the online pre-concentration step allows for low detection limits of these compounds without the time consuming steps of offline solid phase extraction of 1L sample volumes.

Novel aspects:
High resolution mass spectrometry plus online pre-concentration to screen, quantify and confirm 27 antibiotics in water samples at low ppb levels

No.100514

# Rapid screening and confirmation of emerging contaminants in UK river waters by UHPLC-IT-TOF

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Keywords: river water screening environment I.T-TOF

The extensive environmental distribution of emerging contaminants and their potential ecotoxicological effects at very low concentrations has attracted increasing interest amongst researchers, regulatory authorities and the public worldwide. Emerging organic contaminants constitute a diverse set of compounds including pharmaceuticals and personal-care products (PPCPs), drugs of abuse and their metabolites, polar pesticides and their degradation/transformation products, perfluorinated compounds (PFCs) and organophosphorous flame retardants. The attention given to these contaminants is warranted as, amongst a number of reasons, many of these contaminants are relatively small molecules which may not be effectively removed during drinking water treatment.

Typically multi-residue methods by tandem mass spectrometry provide routine analysis in identifying emerging contaminants due to their sensitivity and selectivity. These are however limited to unit resolution and have low sensitivity in full scan. For these reasons the employment of high resolution mass spectrometry has become increasing popular in environmental analysis using full scan. Time of flight technology offers the possibly of accurate mass and isotope distribution analysis of targets compounds, however without specialist software, typically data analysis can be labour intensive.

In this study we address the discussed challenges by presenting a fast screening and confirmation method using UHPLC-IT-TOF (Shimadzu Corporation) with software (MetID, Shimadzu Corporation) to aid in rapid and relatively straightforward interpretation of large data files.

River water was collected from a major river in the UK and screened for the presence of over 100 emerging organic contaminants. Samples were extracted using Oasis HLB cartridges. UHPLC separation was achieved using a Kinetex XB-C18 100 x 2.1mm column, with 0.2% formic acid in both the aqueous and organic (methanol) phases, and separated by binary gradient over 25 minutes. LCMS-IT-TOF data was acquired in the mass range 70-900 Da in both positive and negative ionisation modes using fast (100ms) polarity switching.

Preliminary data indicated several organophosphorous flame retardants were detected in addition to pharmaceutical compounds and an insect repellent compound. With these preliminary identifications, comparison to authentic standards will enable final confirmation without the expensive task of purchasing authentic standards for all possible screened compounds.

Novel aspects:

Presented is a fast screening and confirmation method, applied to UK river waters, using UHPLC-IT-TOF with software (MetID) to aid in rapid and relatively straightforward interpretation of large data files

No.100525

# Degradation of Pentachlorobenzene by Fungi Screened from Nature

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Keywords: degradation, pentachlorobenzene, white rot fungi, metabolite

**Introduction:** Pentachlorobenzene is used as an intermediate in particularly the fungicide pentachloronitrobenzene. It has also been used as fire retardant.This compound was added in 2009 to the list of POPs compounds covered by the Stockholm Convention. Considering pentachlorobenzene potential negative effects, it is necessary to address the environmental persistence of this pesticide and to develop effective methods for remediation by miroorganisms. White rot fungi are well known for their outstanding ability to produce extracellular oxidative enzymes e.g. lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase, which are involved in the degradation of either lignin than a wide range of pollutants include pentachlorobenzene. Other non-ligninolytic enzymes may participate in the transformation of pentachlorobenzene. The objective of these studies is to screen the fungi from nature having ability to degrade pentachlorobenzene on solid medium and in liquid medium, to investigate the enzyme activities secreted by selected fungi and to investigate the major metabolic products.

**Experiment:** Fungi screened from nature were inoculated for 7 d on a malt extract agar medium containing 1 mg pentachlorobenzene. Liquid culture experiment was conducted using 20 ml of malt extract medium in a 100 ml Erlenmeyer flask. After inoculation fungus for 7 d, 0.1 mM pentachlorobenzene solution was added and then incubated for 15 and 30 d. After a period of culture, the sample was extracted using ethyl acetate and purified using silica gel column chromatography eluent with hexane:dichloromethane (3:1). The concentrate dissolved in toluene was analyzed by GC-MS Shimadzu QP-2010 equipped with a TC-1 column (30 m, id: 0.25 mm). The carrier gas was helium delivered at a constant flow rate of 1.5 ml/min with column pressure of 100 kPa and interface temperature of 120 ° C. The temperature program was started at 120 ° C for 1 min, raised 20 ° C/min to 180 ° C, then 2 ° C/min to 210 ° C, then 5 ° C/min to 310 ° C, and maintained at 310 ° C for 3 min to allow the eluting peak to exit the column. For enzyme activity analysis, after period culture, the extracellular fluid was collected, filtered through a 0.2 μm membrane filter and measured by using Spectrophotometer. Enzymes that checked were laccase, MnP, LiP, 1,2 dioxygenase and 2,3 dioxygenase. For investigation of metabolic products, the samples were extracted with ethyl acetate and then the identification of metabolic products was performed in comparison with authentic standards.

**Results and discussion:** Cultivation of 4 fungi screened from nature on solid medium containing pentachlorobenzene indicated that growth of the fungi was obtained maximum of 100% growth by 10th to 15th d. On solid medium, U80 showed the most-degrading pentachlorobenzene fungus. In liquid medium, U80 degraded pentachlorobenzene at 63% on 30 d. All enzymes tested were produced by U80 during pentachlorobenzene degradation which the high enzyme activity were obtained in dioxygenase and LiP. By comparing retention times and mass spectra with standard compounds by GC/MS, U80 produced 5 metabolite products. Pentachlorobenzene was initially dechlorinated to form tetrachlorobenzene, which was converted to trichlorobenzene, dichlorobenzene and chlorobenzene. Dechlorination was occured by presence of LiP. Moreover, dichlorobenzene could form 4-chlorophenol or 2,4-dichlorophenol. Dichlorophenol could be identified as a metabolite from dichlorobenzene or a product of dichlorobenzene dihydrodiol decomposition. It was caused by monooxygenase or dioxygenase because the initial attack on dichlorobenzene at the 1 or 2 position could form 4-chlorophenol or 2,4-dichlorophenol.

Novel aspects:
Limited information about degradation of pentachlorobenzene, a newly POPs, by white rot fungi and its metabolite products

No.100526

# Analysis of thermal products of chlorpyrifos using LC/FTMS

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Keywords: chlorpyrifos, thermal products, LC/FTMS, isomers containing chrlorine

Introduction

Chlorpyrifos (O,O-diethylO-3,5,6-trichloropyridin-2-yl phosphorothioate) is an organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. Chlorpyrifos have been used in the lawn on a golf course. It was used for termite control as chlordane alternatives. Sakiyama *et al.* have reported that 2,3,7,8-TCDD- N-analogue is formed in pyrolysis experiments of chlorpyrifos by GC/HRMS analysis.[1] We should consider carefully burning scrap woods of houses contaminated by chlorpyrifos. It is suggested that the influence appears. In this study, the further thermal products are examined by LC/FTMS and the thermal degradation pathways are proposed.

Methods

All pyrolysis experiments were carried out in sealed brown glass ampoules (10 ml) with about 2 mg of chlorpyrifos and 3,5,6-trichloro-pyridinol (3,5,6-TCP) at temperature between 300 °C and 380 °C. After cooling to room temperature, the ampoules were opened carefully and the reaction products were extracted with toluene. The toluene was concentrated to 1 ml under gentle nitrogen stream. The thermal products of chlorpyrifos and 3,5,6-TCP are analyzed with an Exactive orbitrap mass spectrometer(ThermoFisher Scientific Inc.) equipped with positive electrospray ionization(ESI) probe and Accela LC system (ThermoFisher Scientific Inc.) with a column of Inertsil ODS-3 5um 2.1×250mm (GL Science). The best LC separation for thethermal products was achieved using (a) ammonium acetate aqueous solution (5mM) and (b) acetonitrile for the gradient conditions.

Result and discussion

Analysis of chlorpyrifos after thermal treatment at 300 ~ 380 °C was performed by LC/FTMS.The peak intensity of 3,5,6-TCP (m/z= 195.91) was found to increase with decrease in that of chlorpyrifos. The results indicated that the formation of 3,5,6-TCP was probably due to the heating of chlorpyrifos.In addition, the thermal treatment of 3,5,6-TCP showed that the intensity of dimer, trimerand tetramer ions increased as the peak intensity of 3,5,6-TCP decreased in the mass spectra. The analysis of separation behavior for the LC-MS chromatogram indicated that these polymeric species contained some structural isomers. The new finding obtained from the present LC/FTMS measurements is that the heating of chlorpyrifos generates its oligomers by polymerization. The oligomers are shown to have a wide variety of isomers with different binding sites of chlorine atoms.

1)Sakiyama T, Weber R, Behnisch P, Nakano T, PreliminaryAssessment of Dioxin-Like Compounds In/From Chlorpyrifos - A PotentialPrecursor of the Pyridine Analogue of 2,3,7,8-TCDD, Organohalogen compounds: Vol. 73, 146-149 **(2011)**

Novel aspects:

The thermal products of chlorpyrifos were identified to be its polymeric species containing a wide variety of structural isomers with different binding sites of chlorine atoms.

No.100589

# Method development for simultaneous analysis of hydroxylated polychlorinated biphenyl by GC-ECNI/MS in biota sample

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Keywords: hydroxylated polychlorinated biphenyls, biota samples, GC-LRMS (ECNI), isotope dilution

Halogenated phenolic compounds are known to be strongly retainedin human and wildlife blood. Among those compounds, a large number of hydroxylated polychlorinated biphenyls (OH-PCBs) have earlier been reported in blood from humans, terrestrial and marine mammals.

Some analytical methods, which employed mainly gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) using electron ionization mode (EI) have already been applied for OH-PCBs analysis. Although GC-HRMS(EI) can provide high sensitive and selective data, the instruments are extremely expensive for routine and high through-put analysis and require advanced technical skills for operation. A gas chromatography coupled to low-resolution mass spectrometry (GC-LRMS) with electron capture negative ionization mode (ECNI)is widely used analytical instruments in environmental chemistry.

The objective of the present study is to develop the fast and high sensitive methods for the analysis of OH-PCBs encompassing a wide range of homologues in blood sample by isotope dilution method using GC-LRMS (ECNI) analysis.

In biota sample, extracts of human serum (2-3 g) and whole blood (10g) samples from human and wildlife were analyzed. Briefly, blood samples were denatured with 6M HCl and 2-propanol, and extracted with 50% methyl t-butyl ether (MTBE)/hexane. $^{13}C_{12}$-labeled PCBs as well as OH-PCBs were spiked as internal standards. The organic phase was partitioned intoneutral (containing PCBs) and phenolic (containing OH-PCBs) fractions with KOH(1M ethanol/$H_2O$, 1:1, v/v). The alkaline phase(containing OH-PCBs) was acidified to pH 2 with sulfuric acid and re-extracted with MTBE/hexane. OH-PCBs fraction was passed through non-activated silica-gel column chromatography. OH-PCBs were derivatized with trimethylsilyldiazomethane. The derivatized solution was further cleaned up by using activated silica-gel column. Identification and quantification of MeO-PCBs were analyzed by GC-LRMS (ECNI) and compared to GC-HRMS (EI).

Firstly, we compared the instrumental detection limit (IDL) of OH-PCBs in GC-LRMS (ECNI) and GC-HRMS (EI). The IDL was defined as 3 times the standard deviation (SD) of 5 replicate injections of a low concentration standard solution of OH-PCBs (1 pg $ml^{-1}$). The IDLs were 4·OH-triCB29 (22 pg), 4·OH-tetraCB79 (14 pg), 4OH-pentaCB107 (0.65 pg), 4OH-hexaCB146 (1.6pg), 4OH-hexaCB159 (0.90 pg), 4·OH-hepta-CB172 (0.81 pg),4OH-heptaCB187 (0.57 pg) and 4OH-octaCB201 (0.63 pg) by GC-ECNI-MS analysis, andcompared to 4·OH-triCB29 (0.82 pg), 4·OH-tetraCB79 (0.56 pg), 4OH-pentaCB107 (0.34 pg), 4OH-hexaCB146 (0.24pg), 4OH-hexaCB159 (0.23 pg), 4·OH-hepta-CB172 (0.40 pg),4OH-heptaCB187 (0.20 pg) and 4OH-octaCB201 (0.52 pg) by GC-HRMS (EI). The sensitivity of penta- to octa-OH-PCBs determined using the GC-LRMS(ECNI) was in agreement with the GC-HRMS (EI) method. However, sensitivities of GC-LRMS (ECNI) to tri- to tetra- OH-PCBs of GC-LRMS(ECNI) were lower than of GC-HRMS (EI).

The recoveries of $^{13}C_{12}$ labeled OH-PCB inblood samples were 61-113 %. The method repeatability was in the range 6.7-10.4% and 0.6-2.5% relative standard deviation (RSD) for the GC-LRMS (ECNI) and GC-HRMS (EI) systems, respectively.

In conclusion, the GC-LRMS (ECNI) and GC-HRMS (EI) systems were found to be equally well suited for determination for OH-PCBs in biological samples at the pg-levels order of penta- to octa-OH-PCBs.

Novel aspects:
The present study is to develop the fast and high sensitive methods for the analysis of OH-PCBs encompassing a wide range of homologues in blood sample using GC-LRMS (ECNI).

No.100613

# Analysis of Metabolites emitted by Soil-Derived Fungi using Ion Mobility Spectrometry based on GC/MS Data Analysis

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Keywords: MVOC; Metabolite; Ion Mobility Spectrometrty; Mass Spectrometry; Cultural Properties

Contaminations of fungi were found on the mural paintings in the famous Takamatsuzuka tumulus in Japan [1]. Fungi often cause serious damage to cultural properties. The purpose of our work is to detect fungal growth by monitoring Microbial Volatile Organic Compounds (MVOCs) emitted from fungi at cultural sites.

Ion Mobility Spectrometer (IMS) is suitable for on location measurements because it is portable. Furthermore, it is a powerful tool to simultaneously detect multiple compounds. However IMS alone cannot be used to identify these MVOCs. In order to identify these compounds, we also must use GC/MS. In this study, metabolites emitted by soil-derived fungi were analyzed using both Ion Mobility Spectrometry and Mass Spectrometry.

Alcohols, aldehydes and ketones were found as MVOCs in most of *Aspergillus fumigatus*, *Aspergillus nidulans*, *Fusarium solani* and *Penicillium paneum*. On the other hand, sesquiterpenes were found in only specific fungi. Because each fungal strain had a characteristic compound (sesquiterpene), these compounds are useful in identifying their respective fungi. As the number of spores increased with the fungal cultivation period, it was found that the amount of these MVOCs, ketones, aldehydes and alcohols also increased. Therefore, 3-octanone is suited to be an indicator of the size of fungi (fungal amounts) because of the positive correlation found between the number of spores and the amount of the compound. On the other hand, sesquiterpenes showed a peak of MVOCs at a particular period right before spore reproduction and therefore are useful to identify both fungal species and their reproduction periods [2].

Ion Mobility Spectra of volatile metabolites emitted from *A. nidulans* were measured using the IMS-MINI Ion Mobility Spectrometer (I.U.T. GmbH, Germany). In the IMS driftgram of MVOCs from *A. nidulans*, a large peak appeared at 6.36ms which correspond to "Reaction Ion Peak" (RIP). From the assignment based on the comparison with GC/MS data analysis of MVOCs from *A. nidulans* cultivated under the same condition as samples measured by IMS; it was found that relatively smaller peaks appeared at 7.69, 8.31 and 9.68ms were assigned to phenyl acetaldehyde, 3-octanone and bisabolene, respectively. A peak at 7.50 ms corresponds to 2-octen-1-ol and 1-octen-3-ol. The IMS driftgram of volatile metabolites emitted *P. paneum* showed the peak corresponding to beta-Caryophyllene. This compound is one of sesquiterpenes and an unique MVOC for *P. paneum.* Therefore, it was concluded that *P. paneum* can be monitored using only a driftgram of MVOC from *P. paneum*.

The IMS drift time for these compounds was also calculated using Monte Carlo simulations.

The calculated drift time was consist with the experiments.

## References

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## Novel aspects:

The microbial volatile organic compounds emitted from fungi may be applicable to a notification of fungal growth in the environment in order to preserve cultural properties using MS or IMS.

No.100667

# Determination of diuretics in urine using immobilized-multiwalled carbon nanotubes hollow fiber liquid-phase microextraction combined with liquid chromatography-mass spectrometry

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Keywords: Diuretics; multiwalled carbon nanotubes; hollow fiber liquid-phase microextraction; liquid chromatography-mass spectrometry

Diuretics, which increase urine flow from the kidneys, are used for treatment of heart conditions, liver,kidney and lung disease, generally to reduce salt or water retention. The Medical Commission of the International Olympic Committee (IOC) included diuretics as a banned substance since 1986. This study was to evaluate a sample treatment technique using immobilized-multiwalled carbon nanotubes (I-MWCNTs) in hollow fiber liquid-phase microextraction (HF-LPME) combined with liquid chromatography-mass spectrometry for diuretics analysis in urine samples. The optimal conditions of sample extraction and mass spectrometry have been studied. The detection limits for the diuretics studied were found to be in the range of 0.19 to 0.96 ng/mL with the relative standard deviation (RSD) below 11.1%. No carryover effect was found, and every laboratory-made I-MWCNTs HF-LPME could be reused for extraction up to 50 times with recovery efficiency above 85%. The I-MWCNTs HF-LPME has been proven effectively for increasing extraction efficiency and reducing matrix interference from urine. The method developed offers not only very high sensitivity for determination of trace diuretics in urine, but also reduced extraction costs for having a long use times of the I-MWCNTs HF. The method is recommended for determination of trace diuretics in urine for its elegance, simplicity and high sensitivity even in the presence of high levels of interference.

Novel aspects:
The laboratory-made immobilized-multiwalled carbon nanotubes (I-MWCNTs) in hollow fiber liquid-phase microextraction (HF-LPME) combined with LC-MS for analyzing diuretics in urine.

No.100687

# DIFFERENTIATION OF FARMED FISH AND WILD SEAFISH USING ISOTOPIC SIGNATURES

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Keywords: Strontium Isotopic Ratios, Light Isotopic Ratios, Farmed VS Wild Marine Fish, Atlantic Cod, Food Fraud

Worldwide consumption of fish has risen dramatically over the decades. Overfishing, a consequence of this demand, has led to decline of fish stocks and depletion of marinefood webs in many parts of the world. To meet the increasing demand for seafood, aquaculture has developed as it offers a cost effective supply of seafood. However, aquaculture employs the use of antibiotics, dyes, growth hormones, "sustainable fish feeds" where marine proteins are being substituted with plantbased proteins as well as fish-feed produced from genetically modified raw materials. This may result in increased levels of contaminants in aquacultured fish but also may change their nutritional value. Wild fish being perceived as the superior product can therefore command a higher price on retail markets. This opens avenues for food-fraud by mislabeling. It is estimated that the origin of 25 to 70% of seafood is fraudulently declared. This project aims to develop an ultrasensitive method to distinguish wild and farmed cod (*Gadus morhua*) by employing strontium ($^{87}Sr/^{86}Sr$), carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios in fish meat as a dietary marker. Techniques involving Thermal Ionisation Mass Spectrometry (TIMS) and Isotope Ratio Mass Spectrometry (IRMS) for measuring carbon, nitrogen and strontium isotope variations were developed.

Differences in natural strontium isotopic ratios arise in nature due to the decay of $^{87}Rb$ to $^{87}Sr$. This radiogenic decay has produced distinctly different Sr isotope abundances in different parts of the Earth crust over its history. This isotopic signature is incorporated into plants and will be passed onto fish through feed prepared from the plant material. In contrast, due to the longresidence time of Sr in seawater (millions of years), compared to the turnover time of the oceans (millennia), $^{87}Sr/^{86}Sr$ is homogeneous throughout the world's oceans at any given time. Wild marine fish should display the unique strontium isotopic ratio of seawater as a result of feeding solely on marine organisms, while farmed fish should exhibit an isotopic ratio more terrestrial in nature, as fish feed in aquaculture utilizes terrestrial plant based materials. Differences in carbon and nitrogen isotopic values arise for the same reason, i.e. wild and farmed cod should differ essentially in the isotope signature taken up from feed.

Methods for the analysis of strontium isotopic ratios in cod involved microwave digestion of tissue, separation of the strontium ions from interfering elements using ion exchange chromatography and finally analysis of the purified fraction using TIMS. Achievable precisions for $^{87}Sr/^{86}Sr$ are of the order of parts per million (0.0017% RSD; n=6). Analysis of both wild and farmed cod from Norwegian fish farms showed similar strontium isotopic ratios despite differences in feed. This contradicts the initial hypothesis that both groups of fish should exhibit different strontium isotopic ratios and that cod acquires strontium primarily from water and not from feed. Measurement precision was on the order of 0.1‰ for nitrogen and 0.06‰ for carbon. By combining both signatures, wild and farmed cod could be distinctly separated from one another which may turn the developed techniques into an important tool for differentiating farmed from wild cod.

Novel aspects:
First report on the use of isotopic tecnniques to differentiate between wild and farmed atlantic cod, particularly the use of strontium isotopic ratios which has never been attempted.

No.100781

# Level of Dechlorane Plus in ambient air and development of monitoring method

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Keywords: Dechlorane Plus, ambient air, NCI

A chlorinated flame retardant, Dechlorane Plus (DP) (*syn*-, *anti*-), was detected and identified in ambient air from Japanese urban area. This study shows that DP was detected in air samples in Japan for the first time. In a moment of this research, we investigated the mode of ionization using GC/MS availability. GC/MS is commonly applying to determine DP with negative chemical ionization (NCI) mode at low resolution (LR). DP was also determined magnetic sector-type MS with electron impact ionization (EI) mode at high resolution (HR). $R^2$ for calibration curves on DP (*syn*-, *anti*-) were over 0.9999 of LR-NCI and HR-EI method. Instrument Detection Limits (IDL) of both methods for DP (*syn*-, *anti*-) were 0.20 pg/$m^3$, 0.23 pg/$m^3$ and 0.34 pg/$m^3$, 0.36 pg/$m^3$ respectively. LR-NCI method was S/N = 50 at 0.1 ng/mL standard solution that approximately four times greater than HR-EI method. Result of compared both methods, LR-NCI method was applying in this study.

Air samples were collected on the rooftop of Japan Environmental Sanitation Center. Samples were collected 24 hours and repeated 4 times. All samplings were implemented duplicate. Result of measurement by GC/MS (LR-NCI), DP air concentration ranged from 1.9 to 21 pg/$m^3$. Recoveries of $^{13}C$ labeled DP were more than 90%. The *syn*- and *anti*- ratio were read between 0.24 and 0.48. All duplicates were considerably fit in each sample. DP concentrations were higher than Mirex to compare with Environmental Survey and Monitoring of Chemicals was implemented by Ministry of the Environment Japan.

Novel aspects:
Dechlorane plus were determined in air samples for the first time in Japan.

No.100787

# Determination of hydroxylated polycyclic aromatic hydrocarbons in mariner's urine by high performance liquid chromatography-tandem mass spectrometry

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Keywords: Polycyclic aromatic hydrocarbons, Urine, Human exposure, LC/MS/MS, metabolite

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals released into the air during the incomplete burning of fossil fuels such as gasoline and other organic substances

Some PAHs are reasonable anticipated to be human carcinogens. The carcinogenic risk to humans among 16 PAHs is classified as probable human carcinogens by the International Agency of Research on Cancer in 2010. PAHs are also known to have endocrine disrupting activity. PAHs are absorbed into the human body through the skin, lungs and gastrointestinal tract and are then metabolized to their hydroxylated PAHs (OHPAHs) and finally excreted in urine. In this study, a high performance liquid chromatography-tandem mass spectrometry method has been developed for the simultaneous quantification of six urinary OHPAHs, including 1-hydroxynaphthalene(1-OHNap), 2-hydroxynaphthalene (2-OHNap), 1-hydroxyphenanthrene (1-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe) and 1-hydroxypyrene (1-OHPyr) in human urine. Deuterated 3-OHPhe-d9 and 1-OHPyr-d9 were used for the quantification of the analyte as internal standards. Considerable amounts of PAHs are present in the workplace. PAHs exposure is reported high in coke plants, aluminium work and paving work. In order to assess the potential health risks posed by exhaust gas from ship and obtain a better understanding of the occupational hazards connected with PAHs exposure, the concentration of OHPAHs in urine collected from mariners has been analyzed.

The urine sample treatment involved enzymatic hydrolysis of glucuronide and sulfate conjugates followed by solid-phase extraction using Sep-Pak $C_{18}$ cartridge for LC/MS/MS analysis. The LC/MS/MS system consisted of an Agilent 1260 infinity series (Agilent Technologies, Santa Clara, CA, USA) and QTRP 5500 mass spectrometer (AB SCIEX, Framingham, MA, USA). The analyte and ISTD were separated from interference peaks on an Ascentis Express C18 column (2.1mmID × 100mm, 2.7 μm particle size; SIGMA-ALDRICH Co., St. Louis, MO, USA). The mass spectrometer was operated under multiple reaction monitoring (MRM) negative mode for the ion transitions m/z 143 → 115(OHNap), m/z 193 →193(OHPhe), m/z 202 →202(OHPhe-d9), m/z 217 → 189(OHPyr) and m/z 226 → 198(OHPyr-d9).

This method was applied to the analysis of OH-PAHs in 29 urine specimens (11 engineers and 18 other crews) collected from the crews on a ship. The average concentrations of urinary1-OHNap, 2-OHNap, 1-OHPhe, 3-OHPhe, 4-OHPhe and 1-OHPyr were 1.6, 2.2, 0.2, 0.2,0.1 and 0.2 μg/g creatinine in engineers and 0.5, 1.8, 0.2, 0.1, 0.1 and 0.1 μg/g creatinine in other crews, respectively. The method can be used to evaluate occupational exposure to PAHs.

Novel aspects:

We assess the potential health risks posed by exhaust gas from ship and obtain a better understanding of the occupational hazards connected with PAHs exposure.

No.100862

# The analysis of olefine and aromatic hydrocarbon in hydrocarbon mixture using Multi-Dimensional GC/GCMS system

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Keywords: hydrocarbon mixture,Multi-Dimensional GC/GCMS system

The component of hydrocarbon mixture is very complicated, including even and odd number carbon hydrocarbon. Its analysis with conventional gas chromatographic approaches is a big challenge and can give us inaccurate or even false results because overlapping is always happened. Many measures were taken to avoid interferences, such as improving sample preparation or using high selectivity detectors. Multi-dimensional GC/GCMS system with multiple heart-cutting is one of the powerful tools. Multi-dimensional GC/GCMS can improve resolution beyond that of the regular GC analysis as it re-introduces the dissolved component of interest into another column. In other words, only part of the peak of the component that was insufficiently separated on the column where the sample initially passed through (called the "1$^{st}$ column") is introduced (heart-cut) to a column of another type (called the "2$^{nd}$ column"), so that insufficiently separated components can be separated. A device called a "switching device" is used for heart-cut introduction of peaks eluted from the 1$^{st}$ column to the 2$^{nd}$ column. As a switching device, the recently developed Multi-Deans switching unit can be used in combination with a GC-FID as the first analytical dimension and a GCMS as the second analytical dimension. The analytes pass the first column and are detected in the FID ("stand-by mode") or are transferred to the second column and analyzed with mass spectrometer or GC detector such as FID ("cut mode"). By using this system, complicated matrix analysis such as hydrocarbon mixture was done to demonstrate MDGC/GCMS system performance.
This MDGC/GCMS system can analyze the olefine and aromatic hydrocarbon in hydrocarbon mixture. It has superiority on the determination of the complicated matrix sample in order to obtain more reliable analytical results.

Novel aspects:
MDGC/GCMS system can show superiority on the analysis of complicated matrix sample such as hydrocarbon mixture, etc.

No.100888

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

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

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

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

No.100973

# A Novel Sensing Material for Iron(III) Ions based on Poly (gamma-Glutamic acid)-grafted-3,4-dihydro-3-2'-ethylhydroxyl-6-methyl-1,3,2H-benzoxazine

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Keywords: Poly(gamma-glutamic acid), benzoxazine, Fe(III) ion, colorimetric sensing material, photometric titration method

A novel sensing material for Fe(III) ion was prepared from poly(gamma-glutamic acid) (gamma-PGA) and 3,4-dihydro-3-(2'-ethylhydroxyl)-6-methyl-1,3,2H-benzoxazine (Mt-Bx). Mt-Bx was used as an ionophore segment and grafted onto the gamma-PGA backbone via an esterification reaction. The optimum reaction time determined by FT-IR was 2 h. The calculation based on $^{1}$H-NMR spectrum revealed that the most attainable grafting degree was 30%. This copolymer showed a highly selective and sensitive recognition toward Fe(III) ions. A simultaneous transition of color and solubility was observed when the copolymer formed complex with the Fe(III) ions. These responses were clearly observable with the naked eye. A quantitative analysis based on a photometric titration method indicated that the copolymer exhibited and excellent interaction with Fe(III) ions at a stoichiometric ratio of 1:390.

Novel aspects:
The novel polymeric sensing materials was synthesized based on a simple reaction. The responsiveness of the polymer toward Fe (III) is very rapid, and clearly observable with the naked eye.

No.100471

# High-Resolution Tandem Mass Spectrometry Analysis of the Interactions of Oligonucleotides with Selected River Basin Specific Pollutants

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Keywords: Oligonucleotides, organic micro-contaminats, high resolution tandem mass spectrometry, micro-flow liquid chromatography, non-covalent interactions

Since a great number of organic compounds are annually released into the environment, the necessity to assess in a timely manner the potential risks associated with these chemicals along with the products of their environmental transformation is of high priority. In order to reduce the number of candidates for full-scale animal studies, potential toxicity of the compounds of interest can be rapidly assessed in simplified model systems. In this study we employed high resolution electrospray ionization tandem mass spectrometry (ESI-TOF/MS/MS) along with micro LC-ESI-TOF/MS to study interactions of selected river basin pollutants with model nucleic acids. The micro-contaminants were chosen in the context of the European Union Water Framework Directive (EU WFD) based on the assessment study for 500 organic substances observed in the four European river basins of the Elbe, Scheldt, Danube and Llobregat.

Two AB SCIEX LC-MS systems were used in this study: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument with Turbo V™ sources and Electrospray Ionization (ESI) probe in the negative ion mode. A high pressure micro-flow LC system was utilized for chromatographic separation. Interactions of two decameric oligonucleotides (ODNs), d(5'-GCGCATGCGC-3') and d(5'-GCGCGCGCGC-3') with diazinon, diuron, alachlor and bis(2-ethylhexyl)phthalate were investigated in the direct infusion mode and with the micro-flow LC separation. The AB SCIEX PeakView™ software with a prototype oligonucleotide fragmentation interpretation tool was used for the data analysis.

In this study we examined the interactions of two self-complementary decameric ODNs with four river basin specific pollutants. ESI-MS/MS analysis of the incubation mixtures of diuron with two selected ODNs indicated the formation of 1:1 adducts of both single-stranded oligonucleotides at a molar ratio of 10 or higher. Collected high resolution MS and MS/MS data were used to confirm the elemental composition of the ions of interest as well as to gain an insight into the structure of the adducts.

Tandem mass spectrometric measurements of the 1:1 adducts of diuron with both single-stranded ODNs demonstrated that their dissociation proceeds via the loss of a neutral diuron molecule at a relatively low value of the laboratory frame collision energy. When highly negatively charged diuron:ODN adducts (charge states of 5-, 6- or 7-) were exposed to the collision-induced dissociation (CID) we have also observed a competing charge-separation channel to produce a deprotonated diuron moiety and an oligonucleotide ion in (n-1)- charge state with n=5,6,7 respectively. Based on this observation we hypothesize a non-covalent mode of binding in the 1:1 complexes. Under the studied experimental conditions we did not detect diuron adducts with the ODN duplexes.

The results of the experiments performed with the studied ODNs and diazinon were similar to those observed for diuron-containing species. However, the formation of the 1:1 adducts was observed at a higher excess of diazinon (50-fold or higher). CID measurements of the diazinon adducts demonstrated an earlier (compared to diuron) CID onset which indicates a weaker binding with the ODNs. No detectable adducts of alachlor with either ODNs were formed even at 1000-fold excess of the compound.

This study has demonstrated an elegant application of high resolution tandem mass spectrometry combined with the micro-flow liquid chromatography for rapid screening of the reactivity of high priority environmental contaminants toward DNA models.

Novel aspects:

For the first time the reactivity of selected organic micro-contaminants towards oligonucleotides was assessed using high resolution electrospray ionization tandem mass spectrometry combined with the micro-flow liquid chromatography.

No.100637

# Proteomic analysis of low temperature resistant system in bacteria

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Keywords: Bacteria, cold shock resistant system, nano LC-MS/MS

Under the cold condition, the increased production of cold shock proteins (CSPs) is mainly a consequence of the increased stability and preferential translation of the corresponding mRNAs. This response, which in general terms in similar in different mesophilic bacteria, helps to counteract a number of stresses derived from low temperatures such as reduced membrane fluidity, reduced enzyme activity, and the greater stability of DNA and RNA secondary structure that can diminish the efficiency of replication, transcription and translation. The bacteria stay long time under low temperature, the growth of cell was stopped. After stopped growth, temperature returned $4^{o}$C to $37^{o}$C, the cells be able to grow normally. Usually, the knowledge of cold shock response system was obtained from model-bacteria (*Escherichia coli* and *Bacillus subtilis*) and $15^{o}$C. But most model-bacteria cannot grow at under $16^{o}$C. Most studies on the biology of bacteria have been performed on cells grown at $30^{o}$C, its optimum growth temperature. However, when growing in natural habitats, bacteria frequently live much low temperature place that appears under $10^{o}$C and occupies 70% of the earth. *Pseudomonas putida* F1 is model bacteria living in soil, this family was isolated from the iceberg of the south antarctic pole. Because, we think that the genome of this strain was included essential genes of constant low temperature growth.

The cold shock system is suggested that it is the stress response protein to rescue from inconvenient environment of low temperature, and the possibility that there is not the protein necessary to normal grow under the constant low temperature. This present works report proteomic analysis comparing two conditions, the one is $30^{o}$C, $16^{o}$C and $4^{o}$C, and the second is cold shock stress phase (CSSP) and constant low temperature phase (CLTP).

To obtain the low temperature resistant system of bacteria, we prepared three kind of the temperature, $30^{o}$C is optimum growth temperature, $4^{o}$C is low temperature that almost model-bacteria cannot grow under this condition, $16^{o}$C is intermediate temperature previous two conditions. *P. putida* F1 was cultured at its optimum growth temperature in minimal salt medium. After grew at exponential phase (O.D.600 = 0.3), all proteins were extracted from cell. The proteins were separated by SDS-PAGE, and gels were cut into about 60 slices for in-gel digestions by trypsin. Each peptide mixture was analyzed by nano LC-MS/MS (Thermo Fisher Scientific, USA) for protein identification. The LC-MS/MS data were searched by the *P. putida* F1 database in NCBI, and, expression level of each gene were appeared by emPAI value calculating from Mascot program (ver. 2.3.01).

About 2000 proteins were identified based on more than two unique peptides. The number of proteins expressed under several temperatures was about 40% of all proteins (5250). The overview of expression genes was appeared to compare protein expression at $30^{o}$C. In $16^{o}$C, a group of 340 genes displayed a two-fold significant increase at CSSP, and 789 genes displayed a two-fold increase under the phase of the CLTP. In $4^{o}$C, the numbers of CSSP genes are 330, and CLTP genes are 1051. The interesting result in this, almost genes that showed expression more than two-fold were phase-specific. The expression increase genes were separated about function, we don't find the common genes of low temperature (CSSP vs CLTP and $16^{o}$C vs $4^{o}$C). When sigma factor (RNA polymerase subunit) expression was checked in several conditions, that expression pattern was independently about each phase. RpoS (the homolog gene of *E. coli* control CSPs expression) was only induced in CLTP at both temperatures. This result shows that the mechanism of low temperature resistance is different between the CSSP and the CLTP, $16^{o}$C and $4^{o}$C.

Novel aspects:
This proteomic approach is shown to be ideal as a primary analysis for examination of various physiological characteristics in bacterial cells comparably to a DNA array analysis approach.

No.100828

# Simultaneous determination of cationic and anionic compounds using a high-speed polarity switching ESI and an online-SPE LC-MS/MS

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Keywords: Online SPE LC-MS/MS, high-speed polarity switching, simultaneous determination, phthalate monoesters, phthalate diesters

Mounting evidence suggesting ubiquitous presence of newly or unexpectedly detected chemicals, namely contaminants of emerging concern (CECs, e.g. pharmaceuticals and personal care products: PPCPs, perfluorinated compounds, steroid hormones and others), has lead public concern and government actions. The CECs consist of hundreds of compounds, both cationic and anionic, and exist in the environment in generally trace levels. Those compounds typically enter the environment through sewage systems since the current wastewater treatment technologies are not designed for decomposing CECs, which is considered as an important exposure route for aquatic ecosystem. Some of the CECs are also found to resist drinking water treatment, which could result in human exposure. To establish an effective monitoring system for wastewater influent and drinking water, a high-through-put analytical method is in acute need.

For the analysis of trace level compounds, the current sampling methodologies often involve large volume sample collection to concentrate analytes in pretreatments, which limits sample transportation and introduces complex sample pretreatments. Recent advances in online solid phase extraction (online SPE) technology may be one of the solutions to this limitation since it requires relatively small volume of samples (typically 1-10 mL) and introduces all the injected volume into an analytical system. A newly developed high-speed polarity switching technology is capable of simultaneously determining cationic and anionic compounds including PPCPs, steroid hormones and other CECs.

An online SPE liquid chromatography tandem mass spectrometry (online SPE LC-MS/MS; Shimadzu Corporation, Kyoto, Japan) was used to develop a simultaneous quantitation method for the analysis of phthalate di- and mono-esters (PEs) in finished water. One mL of raw samples was injected after receiving stable isotope labelled PEs. Two binary pumps were used for sample cleaning with an SPE column (MASK-ENV, Chemco Scientific Co., Osaka, Japan) and another two pumps were used for separation by an analytical column (Shim-pack XR-ODS). For both sets of pumps, scrubber columns (Shim-pack XR-ODS) were inserted just after mixing chambers to reduce contaminations from the analytical system.

Method detection limits (MDLs in ng/ml) were as follows: monometyl- (MMP, 0.203), monoetyl (MEP, 0.162), mono-*n*-butyl (MnBP, 0.214), monobenzyl (MBzP, 0.201), mono-2-ethylhexyl- (MEHP, 0.213), mono-2-ethyl-5-hydroxyhexyl- (MEHHP, 0.223) phthalates; dimethyl- (DMP, 0.144), dietyl- (DEP, 0.179), di-*n*-butyl- (DnBP, 1.43), butylbenzyl- (BBzP, 0.117), di-(2-etylhexyl)- (DEHP, not calculated due to blank contamination) phthalates. Recoveries ranged 56%-134%. DnBP and DEHP were detected even in procedure blanks suggesting that contaminations occurred during sample preparations or analytical procedures. Phthalate monoesters were not detected or below MDLs in the most of samples although MEHP was detected in one sample (0.471 ppb). Since this method is capable to detect very low levels of PEs, it is essential to minimize contaminations from sampling to analysis in order to achieve lower MDLs for phthalate diesters. In the presentation, preliminary result of the application of online-SPE-LC-MS/MS system for the measurement of phthalate esters will be discussed.

Novel aspects:
Developed was simultaneous quantitation technique of cationic and anionic compounds which requires small volume of environmental samples and thus enables high through put screening of contaminants of emerging concern.