Electrospray mass spectrometric observation of the interaction between environmental pollutants and biologic compounds (Hiroshi Moriwaki)

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

Suspended solid as a disturbance of PFOS analysis in case of wastewater

Degradation of Pentachlorobenzene by Fungi Screened from Nature

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

Real-time ambient air monitoring using selected ion flow tube-mass spectrometry (SIFT-MS) (Vaughan S. Langford)

Development of UPLC-MS/MS method with large volume injection for simultaneous determination of regulated pesticides in drinking water. (Jun Yonekubo)

Development of Automated Identification and Quantification System with a Database (Kiwao Kodakami)

Metal speciations in environmental and clinical applications (Yeuk-Ki Tsoi)

Occurrence of 92 pharmaceuticals in river water in agricultural or urban areas (Koya Komori)

Application of tandem mass spectrometry for the structure confirmation of a wide range of peptides synthesized by cyanobacteria Woronichinia naegeliana (Beata Bober)

A search for active ingredients in cigarette smoke that modify significant biomolecules (Shizuyo Horiyama)

Development of smoke diagnostic assays: When the smoke clears, will it end up in the wine bottle? (Yoji Hayasaka)

Ions Observed in DART-MS Analysis of Pharmaceuticals Containing Various Functional Groups on Normal and Reverse Phase TLC Plates (Kaori Asano)

Formation of Hydroxy Polychlorinated Biphenyl (Shiho Fukuzawa)

High Resolution LC-MS for Screening and Quantitative Analysis of Antibiotics in Drinking Water using an Orbitrap and Online Sample Preparation (Jonathan R. Beck)

Rapid screening and confirmation of emerging contaminants in UK river waters by UHPLC-IT-TOF (David R. Baker)

Degradation of Pentachlorobenzene by Fungi Screened from Nature (Ajeng A. Sari)

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

Behavior of Hexabromocyclododecane (HBCD) stereoisomers in water, sediment, and biological samples. (Hitomi Hasegawa)

LC/MS/MS determination of hair-dye ingredients in products, water and urine: estimation of human exposure and environmental release. (Mari Takazawa)

Qualitative analysis of waste leachate by using exact mass of LC/Q-ToFMS/MS (Akira Murakami)

Investigation of Perfluorinated compounds in Osaka-bay over past three years (Shusuke Takemine)

Computational Chemistry Study on Negative Ion Chemical Ionization Mechanism of Peroxyacetyl Nitrate (Yasuyuki ITANO)

Analysis of inadvertent PCBs contained in consumer goods (Masanobu Yokota)

Method development for simultaneous analysis of hydroxylated polychlorinated biphenyl by GC-ECNI/MS in biota sample (Eguchi Akifumi)

Suspended solid as a disturbance of PFOS analysis in case of wastewater (Hitomi Oka)

Analysis of Metabolites emitted by Soil-Derived Fungi using Ion Mobility Spectrometry based on GC/MS Data Analysis (Shoko Ichii)

Measurement of brominated flame retardants in the environment near factories by LC/MS/MS. (Itaru Oue)

Determination of sulfonamides and tetracyclines in livestock wastewater using hybrid ion trap - time of flight mass spectrometer (Youngmin Hong)

Dechlorane Plus, a highly chlorinated flame retardant in Japanese environment samples. (Takanori Sakiyama)

Multi-residue method for rapid screening of veterinary drugs in muscle matrices by UHPLC-MS/MS (Insun Lee)

Development of LC-MS/MS method to monitor pharmaceuticals in environmental wastewater (Minhye Lee)

Analysis of Ultraviolet Absorbers in Urine Samples by Functionalized Nanomaterial-assisted Electrospray Mass Spectrometry (Tzung-Jie Yang)
<table>
<thead>
<tr>
<th>ID</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>100972</td>
<td>Analysis of natural organic dyestuffs extracted from textiles.</td>
<td>Ivan Viden</td>
</tr>
<tr>
<td>100107</td>
<td>Dramatically Improved Hydrocarbons Analysis with the 5975-SMB GC-MS with Cold EI</td>
<td>Alexander B. Fialkov</td>
</tr>
<tr>
<td>100193</td>
<td>Mass spectrometric analysis of 1,3,5-Trinitroperhydro-1,3,5-triazine (RDX)</td>
<td>Sehwan Park</td>
</tr>
<tr>
<td>100247</td>
<td>Screening of five mycotoxins by using immunoaffinity column and HPLC-orbitrapMS in processed foods</td>
<td>Dong Sik Jeong</td>
</tr>
<tr>
<td>100292</td>
<td>Untargeted screening of pesticides metabolites by LC-HRMS: a tool for human exposure evaluation?</td>
<td>Emilien L. JAMIN</td>
</tr>
<tr>
<td>100410</td>
<td>Photo ionisation time-of-flight mass spectrometry as a powerful tool for the on-line analysis of tobacco and wood combustion and pyrolysis</td>
<td>Christian Busch</td>
</tr>
<tr>
<td>100455</td>
<td>Determination of DNA adducts originating from methyleugenol using isotope-dilution UPLC-ESI-MS/MS</td>
<td>Wolfram Engst</td>
</tr>
</tbody>
</table>
Dioxin Food Crises and New POPs: Challenges in Analyses

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University of Liege, Liege, Belgium

Keywords: Food; Screening; SectorHRMS; HRTOFMS; GCxGC

When more than a million of broiler chickens suddenly and unexpectedly died in the eastern and midwestern parts of the United States in late 1957, the first dioxin crisis record was set. The 1999 Belgian dioxin chicken gate affair ultimately demonstrated the economic damage such a contamination episode could yield to and pushed the European Union (EU) to set an efficient and pro-active monitoring program to ensure proper quality of the European food and feed web and try to maintain most of the population below tolerable weekly intake. The current European Commission (EC) strategy relies on the implementation of maximum (and action) residue levels (MRLs) for selected polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) (both families actually called 'dioxins'), and dioxin-like polychlorinated biphenyls (DL-PCBs).

To ensure the adequate production of comprehensive and reliable data on the presence of PCDD/Fs dioxins and DL-PCBs in food and feed, a screening-confirmatory approach was thus early adopted for the official control of the PCDD/F dioxins and DL-PCBs. In practice, screening is most of the time performed using chemical activated luciferase gene expression (CALUX) bioassays, response-binding assays (RBAs) based on the aryl hydrocarbon receptor (AhR), although the sole confirmatory method is gas chromatography coupled to $^{13}$C-labeled isotope dilution sector high resolution mass spectrometry (GC-IDHRMS) [1]. More than 10 years after the implementation of the screening-confirmatory approach, the analytical situation has drastically evolved because of advances in automation and hyphenation of parallel sample preparation techniques that allowed both cost and result delivery time to be significantly reduced (down to 350 EUR and 24h, respectively) for the confirmatory GC-IDHRMS method.

Nevertheless, RBAs could and should now be used with non-restrictive sample preparation techniques that would allow most toxicants present in the sample to interact with the AhR to give a general persistent organic pollutant (POP) toxicity information rather than an estimation limited to dioxin regulation compliance. This would be much more biologically relevant and would allow to enlarge current food safety practices to other known and unknown POPs. Sample showing high response for the biological screening should be further analyzed in the hope of identifying new compounds. The extension of the list of target compounds to more ‘exotic’ (un)suspected persistent molecules present in our food requires both chromatographic resolution and instrumental limits of detection (iLODs) to be improved.

Using comprehensive two-dimensional GC (GCxGC) [2] or cryogenic zone compression (CZC) [3] GC coupled to HR time-of-flight MS (HRTOFMS) operating in full scan (FS) mode [4] could nicely complement the classical GC-sector IDHRMS performing in selected ion monitoring (SIM) used for target analyses. This would open the possibility to screen for other compounds (organochlorine pesticides, halogenated flame retardants, GC-amenable perfluorinated compounds, ...) than the one under current regulation without compromising sensitivity. Recent advances in coupling between GCxGC and HRTOFMS nicely put this approach one step further as it allows to produce elemental composition data for unknown compounds present in complex mixtures. Furthermore, when considering halogenated compounds, such a system can be granted by a tremendous improvement in sensitivity (back to low fg iLODs) by favoring resonance electron capture (REC) when operating in negative chemical ionization (NCI) rather than electron impact (EI). Major recent analytical advances in the GC-MS of emerging GC-amenable POPs in the context of prioritization of new targets for food control will be highlighted.


Novel aspects:
Comprehensive emerging POP analyses
High-Speed Survey Method for Photo-degradation Products of Pharmaceuticals Using UV-LED Lighting Device and DART-TOF Mass Spectrometer

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Keywords: PPCPs, Photo-degradation, DART-MS, UV-LED, Pharmaceuticals

Over the past decade, attention as organic pollutants in aquatic environments has been gradually increasing to pharmaceuticals and personal care products (PPCPs). A large amount of pharmaceuticals have been released continuously into aquatic environments for more than a century. Thus, there have been many reports about the occurrence of pharmaceutical products in river water, seawater, and wastewater.

GC-MS and LC-MS or LC-MS/MS are used as the most common techniques for determination of pharmaceuticals in aquatic environments. The selected ion monitoring (SIM), the selected reaction monitoring (SRM) and the multiple reaction monitoring (MRM) methods are utilized as the technique for determination of pharmaceuticals in aquatic environment. When these techniques are used for determination of pharmaceuticals, it is required to obtain the information about the specific ions of target pharmaceuticals prior to starting analyses of them. This indicates that these convenient techniques become useless entirely in determination of unknown compounds.

Photo-degradation is suggested to play an important role in elimination of some of PPCPs from surface waters. In fact, the fate of pharmaceuticals has been recognized by solar and ultraviolet irradiation to them. However, there seems to be also a few reports on the determination of photo-degradation products of pharmaceuticals in aquatic environments in spite of taking a large variety of pharmaceuticals.

Most of investigators have performed the photo-degradation studies by sunlight and/or UV radiation using an appropriately devices equipped with a mercury lamp (Hg-vapor lamp) or a xenon lamp (Xe lamp). The radiation intensity is ca. 300 W/m² (daily average) in the case of solar light 1) and 400 800 W/m² in the cases of Hg-vapor lamp 2) or Xe lamp 3) which consume a relatively long radiation time (about 10 hours) to obtain the significant fate of pharmaceuticals. In addition, these radiation conditions require some cooling system to prevent heat affection. If the photo-degradation study can be performed faster and easier, it will become to assess the photo-degradation products readily. Therefore, we developed the very simple and fast method using a UV-LED lighting device and a DART-TOF mass spectrometer 4).

A small size UV-LED lighting device has been available recently for UV cure processing on pinpoint. At present time, there are several devices giving ultra violet light (λ max: 365 nm) and high power radiation intensity (to 9500 mW/cm²) with no heat affection. We used the cheap model with radiation intensity of ca. 4000 mW/cm² (40000 W/m²) and beam diameter of 4 mm (ULEDN-101, NS-Lighting). After preparation of sample solution using methanol and/or acetonitrile (ca. 5 mg/ml), a portion of several micro-L was applied on the reverse-phase type of TLC plate (RP-2 F 254, Merck) using a glass capillary tube and UV light was irradiated for appropriate time (within 120 sec) on the sample spot (diameter: ca. 3 mm). DART-Mass spectrometry was utilized to detect the photo-degradation products on the TLC plate, because the DART ionization provides direct analysis under atmospheric pressure and few fragmentation of molecular ion. In this study, we used DART-SVP100 (IonSence) for ionization of samples and Accu-TOF mass spectrometer (JMS-T100LC, JEOL) for acquisition of mass spectral data.

In this conference, we will present our high advantage method for exploring photo-degradation products of pharmaceuticals by showing the results in the cases of Ibuprofen, Naproxen, Ketoprofen, Indomethacin and Diclofenac which are generally used as a non-steroidal anti-inflammatory drugs (NSAID).

Reference
1) V. Matamoros, et.al., Water Air Soil Pollut., 196, 161-168 (2009)

Novel aspects:
This highest-speed and simplest method will become extremely useful to survey photodegradation products of pharmaceuticals and personal care products (PPCPs) in environment and drug-development research fields.
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.
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 (CIPAHs) 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 CIPAHs 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 CIPAHs and BrPAHs in flue gas and fly ash from municipal and industrial waste incinerators. However, little is known about congener profiles and distributions of CIPAHs 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 CIPAHs and BrPAHs were therefore synthesized in our laboratory. In this study, we measured individual concentrations of 26 CIPAHs 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 CIPAHs 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 \( \Sigma \) CIPAH and \( \Sigma \) PAH concentrations suggests that direct chlorination of parent PAHs is the mechanism of formation of CIPAHs 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 CIPAHs and BrPAHs congeners including unidentified congeners.
Environmental contamination by POPs where investigated in the biota of different Brazilian ecosystems, including the Amazon equatorial forest in the northern part of the country, tropical river basins, coastal zones and some enclosed bays located near densely populated areas. In the Amazon and on tropical river basins, we have focused our work on different kinds of fishes that represent the main protein source for the local population. In coastal zones, we collected samples of several species of marine mammals (e.g.: dolphins and porpoises) as well as one species of tuna (Thunnus albacares), one piscivorous bird species (Sula leucogaster) sampled from 3 (three) different archipelagos and two species of bivalves (Perna perna and Nodipecten nodosus) obtained from aquaculture established inside coastal bays and lagoons. Most of this work was done in cooperation with foreign laboratories under academic cooperation and joint research projects where the fat extracts obtained with soxhlet apparatus and/or pressurized automatic extraction devices (e.g.: Dionex or FMS). After clean-up using acidic silica gel and florisil columns, the main equipment used in the laboratories was the high resolution gas chromatography, operating in NCI (Negative Chemical Ionization) mode and coupled to low resolution mass spectrometer (e.g.: Quadrupole). Among several OCPs, PCBs and the pesticide DDT and its main metabolite DDE were found in all of the edible fishes (e.g.: Prochilodus sp.) samples analyzed in the Amazon and in the tropical rivers may represent a health risk to the local population. Levels of POPs pesticides and PCBs are also present in hair samples taken from this population. Regarding marine mammals, we could identify in coastal dolphins the presence of high levels of POPs on the blubber of the animals, including a whole bunch of organochlorinated pesticides, brominated flame retardants (e.g.: PBDEs) and also moderate levels of dioxins and furans, and in this case, a high resolution mass spectrometer (“magnetic sector”) equipment was used. The overall pattern of contamination is so clear and separate quite well 3 (three) different bays that we can even say the place where the animal lives, only by looking to its POPs results. A pelagic animal tends to have less contamination than coastal animals. Taking in account the work we did on the avian predatory specie, their eggs are also very contaminated near Rio de Janeiro city, with reducing concentrations in birds eggs sampled far from the main land. On the other hand, ocean pelagic fishes like tuna as well as the cultivated bivalves seems not to concentrated high levels of pollutants in their edible tissues, even if they grown in highly impacted ecosystems. This work represents part of the effort and the commitment of different scientists from different Universities in Brazil to keep tuned with the utmost development of this field of work and to produce high quality data in order to help in the protection of tropical environments. Taking this in account, another application of these mass spectrometer techniques by our group is the deposition of legacy and current-use pesticides on the top of mountains and plateau areas, we found that DDT, PCBs, dioxin/furans, several POPs pesticides as well as endosulfan and chlorpyrifos residues are being deposited over these tropical mountains that are mostly located on natural parks. Nowadays, since cattle grazing activities are present in these areas, in order to cope to the GAPs project and also in order to respond to official demands on scientific results, we are presently collecting raw milk from these areas to see if this contamination is also being transmitted to the food chain.

Acknowledgements: CNPq, CAPES, FAPERJ, UERJ and UFRJ for invaluable support on our research activities.

Novel aspects: Using GC-MS, Hot Spots of POPs of Pollution were found in the Amazon, on fishes from tropical rivers and in Coastal Zones of Brazil.
SELECTIVE EXTRACTION OF ORGANOHALOGENS FROM GCxGC-HRTofMS DATA FOR GLOBAL ANALYSIS OF ENVIRONMENTAL AND BIOLOGICAL SAMPLES

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Keywords: direct-sample-introduction, thermal-desorption, exact-mass, POPs, unknown-chemicals

There are various chemicals around us. Some of them cause problems such as environmental pollution or have adverse effects on organisms. As the number of undesired chemicals grows, they become increasingly expensive in terms of time and cost to monitor and count. This can be partly attributed to problems with analytical methods, which require a lot of time and resources, and where a skilled user is needed to measure trace levels of compounds precisely in the presence of a large amount of interference, and because the methods are individually optimized for each target substance. We are developing and improving methods and tools to overcome the problems related to the techniques used for analyzing organic environmental pollutants. We are currently developing a new apparatus consisting of a comprehensive two-dimensional gas chromatograph (GC × GC), which is a high performance technique for the separation of chemical compounds, directly coupled with a quadrupole type tandem mass spectrometer (MS/MS) and a high resolution time-of-flight mass spectrometer (HRTofMS). We have shown that halogenated compounds can be detected comprehensively and selectively in environmental samples by employing a neutral loss scan (NLS) on a GC × GC-MS/MS. However, the sensitivity of the NLS on this instrument was insufficient to detect trace levels of organohalogens and incapable of identifying compounds due to their unit-mass records.

Here, we report our attempts at the selective extraction of a subset from the GC × GC-HRTofMS data in order to detect and identify trace levels of organohalogens. The data was obtained by measuring several environmental and biological samples, namely flyash, soil, sediment, the atmosphere and human urine. For global analysis, some samples were measured without any purification using a 6890GC (Agilent Technologies) with a KT2004 GC × GC system (Zoex) coupled with a JMS-T100GC (JEOL) HRTofMS. A column pair consisting of 5% phenyl/phenyl-methyl silicone and 50% phenyl/phenyl-methyl silicone was employed as the liquid phase of the GC capillary columns for measurements with the GC × GC system.

Prior to data analysis, 5-15 gigabytes (GB) of raw data from the HRTofMS were converted into 0.5-2.5 GB of data in the netCDF (AIA) format. We attempted to extract only the mass spectra of organochlorines or organobromines using the several methods that we developed. As a result, after data analysis under various mass resolution conditions, we achieved the selective extraction of the mass spectra of chlorinated or brominated compounds under a high mass resolution condition that exceeded approximately 10,000 for some samples. In contrast, low mass resolution below 1,000 was unable to extract the organohalogen data effectively. The results of this study show that data obtained with a high-resolution time-of-flight mass spectrometer are valuable for the global analysis of organohalogens, and probably of other compounds if specific data extraction methods can be devised. However, to improve the data extraction performance the deconvolution of peaks and mass spectra is required because many compounds are co-eluted even when using a GC × GC depending on the sample matrix.

Novel aspects:
It is new to extract compounds on demand from a comprehensive GCxGC-HRTofMS data that includes huge number of chemicals without pre-setting targets.
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 ([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.
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 status¹. 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 contaminants². 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 0.25μm thickness. The temperature was programmed from 90⁰C to 315⁰C.

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.

References:

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.
A Sensitive measurement of sucralose and acesulfame in inland and offshore waters around Japan by LC/MS and LC/MS/MS.

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Keywords: sucralose, acesulfame K, environmental water

Introduction: Sucralose and acesulfame K are widely used artificial sweeteners, which are not likely to undergo a chemical transformation through not only natural processes but also biological reactors. Because of increasing consumption of sucralose and acesulfame K, the environmental behavior and the fate of the persistent sweeteners have been a concern. But the data are mostly limited to Europe, in particular northern Europe, and few other areas. Here we report the distribution of the artificial sweeteners in water in and around Japan islands.

Method: River water samples were collected in areas around Lake Biwa and Lake Suwa. Seawater samples between northern Japan and Sakhalin were collected by auto sampling device equipped in a cursing ship. Sample water was prepared around pH 3 for collecting sucralose (log Kow 0.78) by solid phase extraction (SPE). For collecting acesulfame (log Kow -2.3) by SPE, dihexylammoniumacetate (DHAA) was added to the water sample neutralized with ammonium hydroxide. The river water samples were measured by LC/MS using dihexylammoniumacetate as an ion pair reagent in HPLC, and seawater were measured by LC/MS/MS with triethylamine in mobile phase.

Results: Recovery efficiencies of sucralose and acesulfame from river water fortified with the sweeteners (every 5 ng/mL) were 108% on average (RSD 2.4%) and 109% (RSD 6.8%), respectively. Those of seawater fortified with the sweeteners (every 0.1 ng/mL) were 103% on average (RSD 11%) for sucralose and 91% (RSD 5.1%) for acesulfame.

River waters were sampled around Lake Biwa and Lake Suwa. The concentrations of sucralose and acesulfame in the 25 rivers around Lake Biwa were 1 - 960 ng/L (120 ng/L on average) and 18 - 1500 ng/L (220 ng/L on average), respectively. The river of which the sweeteners' concentrations were higher were tended to be higher in the biochemical pollution indexes such as TOC, BOD, COD, total-N, total-P. The concentrations of sucralose and acesulfame in the 5 rivers around Lake Suwa were 0.1 0.59 ng/L (2.4 ng/L on average) and 2.0 3.1 ng/L (0.29 ng/L on average).

Seawater was sampled at 30 sites with GPS data, time and general water qualities. The concentrations of sucralose and acesulfame were 0.2 to 42 ng/L (3.0 ng/L on average) and 0.6 to 69 ng/L (9.0 ng/L on average), respectively. The concentrations in seawater were almost one hundredth of those in river waters. Concentration ratios of sucralose to acesulfame in seawater were varied from 0.01:1 to 11:1, which were almost similar to those in river waters (from 0.01:1 to 13:1). It is suggested that the concentrations between the two sweeteners had almost no relation to each other both in sea water and river water, and the behaviors of the two sweeteners might be different in environmental waters.

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Novel aspects:
comprehensive research of sucralose and acesulfame K in environmental waters in and around Japan island
Real-time ambient air monitoring using selected ion flow tube-mass spectrometry (SIFT-MS)

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Keywords: SIFT-MS Air-Analysis Pollutants VOC Real-time

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a real-time analytical technique that detects volatile organic compounds and certain inorganic gases down to part-per-trillion levels with no sample preparation. These characteristics mean that SIFT-MS can easily be applied to real-time detection of volatile organic air pollutants. This paper presents results from a air monitoring undertaken at Shu-Lin Elementary School in Taoyuan County, near Taipei, Taiwan R.O.C. from 19 to 21 July 2011.

SIFT-MS uses chemical ionization reactions coupled with mass spectrometric detection to rapidly quantify targeted VOCs. VOCs are identified and quantified in real time from whole-gas samples based on the known rate coefficients for reaction of the chemically ionizing species (so-called reagent ions) with the target analytes. The soft chemical ionization used in SIFT-MS yields a smaller range of product ions than is common in electron impact mass spectrometry (as used by gas chromatography mass spectrometry (GC-MS), for example). Hence the need for gas chromatographic separation of the sample is circumvented, speeding sample throughput and providing instantaneous quantitation of VOCs. Use of several rapidly switchable reagent ions to independently quantify target analytes also greatly reduces interferences, markedly increasing the specificity of SIFT-MS versus other whole-gas analysis technologies.

The high-speed analysis provided by SIFT-MS allows the instrument to provide continuous monitoring. Concentration data were extracted from full scan data for 48 compounds, many of which had been previously identified at the site using GC-MS. A number of these compounds exhibited interesting trends over the sampling period, including toluene, C3-alkylbenzenes (e.g. mesitylene), methanol, isopropyl alcohol, acetone and N,N-dimethylmethanamide. Measured concentrations range from sub-ppb to peak levels for methanol of nearly 250 ppb.

Novel aspects:
Real-time monitoring (without sample preparation) for volatile organic compounds in the vicinity of an elementary school over an extended period.
Development of UPLC-MS/MS method with large volume injection for simultaneous determination of regulated pesticides in drinking water.

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Keywords: LC/MS/MS, pesticides, drinking water

Introduction

Rapid and highly sensitive analysis of drinking water is essential for protecting human health and well-being. The assurance of clean safe drinking water has become more critical given the potential of accidental or international contamination, which has increased in recent years. Highly efficient water treatment processes allow for the effective removal of the majority pesticides that have entered water sources, however, drinking water regulations still require testing to ultra-trace concentrations. This requirement has led to multiple approaches for enriching samples before instrumental analysis, with solid phase extraction (SPE) prior to LC/MS/MS a popular choice. In addition to this online pre-concentration and large volume injection, using specialized injection systems have been employed to introduce samples to LC/MS/MS systems. These techniques have been very successful, but can add time, resources, and complexity to analysis.

Cleaner aqueous samples, such as drinking water are highly compatible for direct injection onto a LC/MS/MS system, but large multi-analyte determinations require extremely fast systems with high-sensitive detection. In this study, 30 pesticides which was regulated as Complementary Items by Ministry of Health, Labour and Welfare (MHLW) in Japan, is targeted. The use of large volume direct injection for the rapid, precise, and high-sensitive analysis of these 30 pesticides in drinking water is developed.

Experimental

UPLC conditions

LC System: ACQUITY UPLC H-class, Run time: 10 min.
Column: ACQUITY UPLC HSS C18, 2.1 x 100 mm, 1.8 um
Mobile phase: 0.05 % HCOOH aq. / CH₃OH gradient
Flow rate: 0.4 mL/min. Injection volume: 50 uL

MS conditions

MS System: Xevo TQD
Ionization mode: positive/negative switching
Capillary voltage: 0.5 kV, Source temp.: 150 °C
Desolvation gas/temp.: 1000 L/Hr (400 °C), Acquisition mode: SRM

Results

Sample throughput
Rapid this system separations allowed a high-throughput analysis with all pesticides of interest eluting before 6.5 min and total runtime of 10 min for each sample.
This system was operated with load-ahead enabled. This allows for the next sample to be ready to inject immediately after the previous sample has completed, which helps optimize instrument efficiency.

High-sensitive detection
Detection of pesticides to lower concentrations was achieved using large volume injection into Xevo TQD. Up to 50 uL injection, there are not observed any deterioration of peak shape and separation. Amount of on-column pesticides with large volume (50 uL) injection was 10 times higher than normal (5 uL) injection. Improvement of sensitivity which was performed to increase of injection volume is able to a few or several ppt level detection.
This level of sensitivity allows detection of pesticides to 1/100 of a desired value and is match for requirement of the regulation.

Linearity and Reproducibility
External calibration (point) of target pesticides was performed at concentrations around the regulatory level for each
pesticides. Good linearity was achieved for all compounds analyzed with typical coefficient of determinations ( $r^2$ ) of > 0.99.

According for the procedure of testing pesticides in drinking water, Reproducibility of this method was verified at 4 or 20 ppt concentrations. MHLW testing procedure mentioned in the method accuracy, Coefficient of variation ( CV ) at 1/100 of each pesticides desired value, It should be 20% or less in principle. % RSD of all compounds were within permissible range.

**Conclusion**

Direct injection of large volume samples onto this system eliminated the need for sample preparation prior to analysis. This methods achieved good sensitivity and linearity, reproducibility for all targeted pesticides in regulation purpose.

**Novel aspects:**
Direct injection of large volume samples onto this system eliminated the need for sample preparation prior to analysis. This methods achieved good sensitivity, linearity, reproducibility for all targeted pesticides in regulation purpose.
Development of Automated Identification and Quantification System with a Database

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Keywords: GC-MS, LC-TOF-MS, TIM, Comprehensive analysis

To thoroughly examine environmental pollution by chemicals, a large number of chemicals should be analyzed. However, because it is known that thousands of chemicals exist in the environment, it is very difficult to analyze all of toxic chemicals simultaneously because of the huge cost and the large amount of labor needed using existing methods. In order to solve this challenge, we have developed two Automated Identification and Quantification System with a Database (AIQS-DB) for GC-MS and LC-TOF-MS, which can measure a large number of chemicals without the use of standards.

In the AIQS-DB for GC-MS, GC retention times, calibration curves, and mass spectra of nearly 1000 semi-volatile organic compounds were registered, and the GC retention times of registered chemicals in actual samples were predicted from the retention times of n-alkanes measured before sample analysis. Differences between predicted and actual retention times were less than 3 s, an accuracy that is nearly identical to that obtained by analysis of standard substances.

The reproducibility of quantification was examined on four GC-MS systems in four laboratories using 114 substances with a wide range of physicochemical properties and which are usually difficult to measure with a GC. When the performance of a GC-MS passed the designated criteria, the reproducibility of quantification results of 47 substances was almost the same as that obtained by a conventional method that uses calibration curves prepared before sample analysis. Although the reproducibility of 42 substances was slightly lower than that by the conventional method, the reproducibility is reliable enough except for in cases that require a high reliability. However, the dispersion of results of 25 substances was large under the GC conditions used. The causes that affect the dispersion of quantification results are functional groups and the structure of molecules. The effects of the functional groups are hydroxyl amino > nitro group. The effects of the number of them are multiple > single. The position of them can also have different effects: farther > nearer. In the case of the structure of molecules, the effects are as follows: side chain > straight chain > single benzene > multiple benzenes. In terms of sensitivity, more than 90% of the chemicals in the AIQS-DB could be detected at a sensitivity sufficient for all practical purposes (100 pg or less).

The AIQS-DB for LC-TOF-MS has been constructed according to the same concept as the GC-MS method. In order to measure as a large number of chemicals as possible, the combination of an ODS column and acetate buffer-methanol eluent (pH 6.8) and positive ionization were used. Retention times of chemicals in the AIQS-DB were predicted from relative retention times of internal standards. Differences between predicted and actual retention times were within +/- 30 s as long as LC conditions were constant. From correct prediction of retention times and high-resolution mass spectra obtained by TOF-MS, identification of chemicals can be consistently performed. Quantification values are also constant as long as LC conditions are constant; reproducibility (RSD) was less than 20%, which is slightly worse than the conventional method that calibration curves are prepared just before sample analysis. However, the value of simultaneous measurement of a large number of chemicals compensates for lower accuracy.

Because each chemical in both AIQS-DB systems can be determined in 1 h, hydrophilic and hydrophobic micropollutants in samples can be measured efficiently and inexpensively by using both the AIQS-DB systems, and to which new substances can easily be added.

Novel aspects:
Novel GC-MS and LC-TOF-MS databases that can identify and quantify micropollutants in environmental and food samples without the use of standards are reported.
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 indispensable 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.


Novel aspects:
high-throughput hyphenated instrumentation; SPME-LC for speciation, modified material for sample preconcentration and simultaneous speciation; selectivity modulation using ion-imprinting technique
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 4.6 km² (St.1), 4.1 km² (St.2), 3.9 km² (St.3) and 5.4 km² (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 sewerized 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/km² of St.1, 99 persons/km² of St.2, 6,594 persons/km² of St.3 and 4,661 persons/km² of St.4.

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

We identified a total of 48 substances (e.g., anti-inflammatory drugs, anti-epileptic drugs, antihypertensives, antibiotics, etc.) in river waters in agricultural and urban areas. The observed average concentration of pharmaceuticals ranged from 0.6 ng/L to 14 ng/L, 0.2 ng/L to 52 ng/L, 0.2 ng/L to 670 ng/L and 0.4 ng/L to 1,500 ng/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.
Application of tandem mass spectrometry for the structure confirmation of a wide range of peptides synthesized by cyanobacteria Woronichinia naegeliana

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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 acute 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 \textit{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 \textit{W. naegeliana} with a high degree of specificity.

Method
Field samples of \textit{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 \textit{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.
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 in cigarette smoke and functional biomolecules.

The purpose of this study is to search for biomolecules modified by active ingredients in cigarette smoke. We tried to identify the products formed by reaction of gas-phase cigarette smoke extract (CSE), from which nicotine and tar had been removed, with L-tyrosine (Tyr) on the assumption that Tyr is a key target amino acid in proteins for cigarette smoke toxicity. A highly sensitive LC/MS/MS system was utilized for detection and identification of trace amounts of these 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 mainstream of smoke (gas phase) from which the particulate phase including tars and nicotine had been removed 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 ºC 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 (Waters Co., 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 ºC) 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, Mr 251 (Tyr+70).

Next, to identify and quantify the compound of Mr 251 (Tyr+70) in CSE we used GC/MS analysis. We found that two peaks indicate Mr 251 from m/z 70 chromatogram of CSE. From the result of the library search of their mass spectra, we identified the peak for tR 9.5 min as crotonaldehyde and the peak for tR 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 ºC. 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.
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.100333

Ions Observed in DART-MS Analysis of Pharmaceuticals Containing Various Functional Groups on Normal and Reverse Phase TLC Plates

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Keywords: TLC-MS, DART, Pharmaceuticals, PPCPs

Thin Layer Chromatography (TLC) is one of the most popular chromatographic methods and widely used as separation and purification techniques in a variety of fields such as pharmaceutical science, forensic medicine, organic synthetic chemistry, botanical science and environmental analysis. TLC is an inexpensive and easy operation method in comparison to high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). However, the identification of compound by TLC analysis often becomes difficult because of its low specificity. To dissolve this problem, mass spectrometry (MS) has been generally used for identification of compounds separated on a TLC plate. In the previous time, the structural elucidation by MS analysis has been carried out after extraction of the target compound from a TLC plate. This off-line TLC-MS method is the relatively much time-consuming technique. Thus, various ionization modes have been investigated to analyze the compounds on TLC plate directly, so far the following ionization techniques have been utilized in a direct sampling TLC-MS analysis; Fast atom bombardment ionization method (FAB), Matrix associated laser desorption ionization method (MALDI), Electrospray ionization method (ESI), Desorption electrospray ionization method (DESI), Direct analysis in real time ionization method (DART), and so on. 1)

Among the direct sampling TLC-MS analyses, TLC-DART/MS seems a relatively easy method if a target compound is not non-volatile, because the ionization of compound on a TLC plate can be performed under atmospheric pressure without solvents and matrixes. However, it is relatively difficult and inconvenient to cut a glass type of TLC plate to a narrow width strip (below ca.10mm) after separation of samples on a TLC plate. A new type of DART ion source was introduced in 2009, which allowed the angle of DART gas stream to be adjusted. The new model shall make it easy to access to more wide surfaces compared with the conventional DART ion source (horizontal type). 2)

On the other hand, the only protonated molecules ions have been discussed in the majority of publications on DART mass spectrometry. In the case of TLC-DART/MS, there are few publications about the detailed investigation on DART mass spectra of compounds containing different functional group. 3) Therefore, we started investigation about the characteristics of the DART mass spectrometry to use the TLC-DART/MS method for analysis of pharmaceuticals and personal care products (PPCPs).

At the first time, we selected the typical pharmaceuticals containing various functional groups, which occurred as the major PPCPs in aquatic environments. Most of compounds were obtained by extraction with methanol from the ethical pharmaceutical products, if necessary, were purified by re-dissolving with acetonitrile and/or solid-phase extraction. The silica gel 60 F254 and RP-2 F254 were used as normal-phase and reverse-phase TLC plates, respectively. Both of them were a glass type of TLC plates (20 X 20 cm, stationary-thickness: 0.25 mm) purchased from Merck. The glass strips (100 mm X ca.10 mm) were prepared by cutting TLC plates and washed by development of methanol in a glass developing chamber. After the sample solutions were prepared in a concentration level of 0.1 to 5 mg/mL using appropriate solvent, a portion of 1 or 2 μL was applied on a TLC plate using a glass capillary. DART-MS system consisted of DART-SVP-100 (IonSence) for ionization of samples and Accu-TOF mass spectrometer (JMS-T100LC, JEOL) for acquisition of mass spectral data.

In this conference, we will present the information on DART mass spectrometry of pharmaceuticals spotted on normal- and reverse-phase TLC plates.

Reference

Novel aspects:
This report is the first detailed study on DART mass spectrometry of pharmaceuticals containing various functional groups in TLC-DART/MS.
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 blubber 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 ¹³C-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 two organs. It seemed that each organ had specific accumulative property of PCB congeners. The total PCB concentrations (ΣPCBs) of males in the liver and blubber were 3,100–1,300 ng/g-fat and 3,000–830 ng/g-fat, respectively.

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

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

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

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.
Investigation of metabolites formed during activated sludge treatment of clarithromycin

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Keywords: clarithromycin, wastewater, activated sludge treatment, metabolite

The occurrence of antibiotics in aquatic environments is of ecotoxicological concern because of potential ecosystem alteration. Prolonged exposure to low doses of antibiotics leads to the selective proliferation of resistant bacteria, which could transfer the resistance genes to other bacteria species. Since most antibiotics used in human treatment are eventually disposed of with wastewater, wastewater treatment plants play a key role in removing antibiotics and preventing them from reaching the receiving water bodies.

Clarithromycin (CAM) is a half synthetic macrolide antibiotic which is induced from the antibiotic erythromycin. In Japan, CAM has been detected at low microgram / liter in some untreated wastewater samples. It is understood that CAM is one of the drugs that has ecological risk in aquatic environments. In most of the investigations reported to date, the efficiency of CAM removal during wastewater treatment is determined by measuring the disappearance of the parent compound. But little attention has been given to the identification and quantification of transformation products formed during wastewater treatment.

In the present study, the activated sludge treatment that was the typical wastewater treatment method was reproduced in the laboratory, and screening of major metabolites of CAM was conducted. To investigate the microbial degradation of CAM in the aeration tank, a batch reactor (5 L) loaded with freshly collected activated sludge from the municipal wastewater treatment plant was spiked with the parent compound at a concentration of 20 μg/L. Negative control experiments (without CAM, with activated sludge) and abiotic experiments (with CAM, without activated sludge) were also performed. For the screening of metabolites of CAM using the liquid chromatography / tandem mass spectrometry (LC/MS/MS) system equipped with an electrospray ionization (ESI) source, the samples were pre-concentrated by solid-phase extraction.

Analysis of samples from batch cultures with activated sludge revealed the formation of a metabolite with m/z 828 (retention time of 15.70 minutes) using LC / (+) ESI-MS [corresponding to protonated molecule (M+H)⁺]. This metabolite was not observed in negative control and abiotic samples, suggesting that this peak is unique to the biotransformation of CAM in the presence of activated sludge. The protonated molecule of this metabolite (m/z 828) is hereinafter termed as M828. Because CAM was detected at m/z 748 (retention time of 15.35 minutes, hereinafter termed as P748) as the protonated molecule (M+H)⁺, M828 increased 80 units more than P748. To facilitate identification of M828, fragmentation studies on P748 and M828 were performed by LC / (+) ESI-MS/MS. A minimum fragment ion to which 80 units shifted in the fragment ion of M828 compared with the fragment ion of P748 was m/z 238. This fragment ion corresponds to fragment ion m/z 158 of P748. There is a possibility of causing the shift of 80 units in M828 with desosamine because fragment ion m/z 158 of P748 is derived from desosamine, which is one of the sugars that composes CAM. Results from the analysis of LC / (-) ESI-MS reveal that m/z 826 was detected only with the sample from the batch culture with activated sludge [corresponding to deprotonated molecule (M-H)⁻]. The deprotonated molecule of CAM was not detected. Hence m/z 826 in a negative ionization is presumed to be a deprotonated molecule of a metabolite of CAM, which corresponds to M828 in a positive ionization. A negative ionization is often caused by the elimination of protons in the acid group. One possible biotransformation that causes the change of +80 units is sulfate conjugation. During activated sludge treatment, sulfate conjugation that is one of the phase II reactions observed as mammal’s drug metabolism may occur in CAM.

Novel aspects:
During activated sludge treatment, sulfate conjugation that is one of the phase II reactions observed as mammal’s drug metabolism may occur in CAM.
Structural Analysis of Cyclodextrin Inclusion Complexes Formed in Nonpolar Solvents

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Keywords: cyclodextrin, inclusion complex, nonpolar solvent, structure

Cyclodextrins (CDs) have played a crucial role in various fields including supramolecular chemistry and analytical science due to their unique properties to form inclusion complexes with a great variety of molecules. However, inclusion complex formation with CDs has been limited to aqueous media and several kinds of polar organic media. On the other hand, the effective guest inclusion into the CD cavity in nonpolar media has not been achieved yet, possibly because the main driving force for the guest inclusion is hydrophobic interactions and/or van der Waals interactions between the guests and the CD cavity, and thus the enormous amounts of nonpolar solvents become a strong competitor for inclusion within the CD cavity. Recently, we found that 6-0-modified beta-CD formed inclusion complexes with polychlorinated aromatic compounds in nonpolar media including benzene and cyclohexane, possibly through the dipole-dipole interactions between the CD cavity and the guest molecule, as well as the spatial fit of the guest into the CD cavity. In this presentation, we report the inclusion complex formation between 6-0-modified beta-CD and various aromatic guests in nonpolar media, and the structural analysis of the resulting inclusion complexes by mass spectrometry.

Heptakis(6-0-trisopropylsilyl)-beta-CD (TIPS-beta-CD) and heptakis(6-0-tert-butyldimethylsilyl)-beta-CD (TBDMS-beta-CD) were used as CD hosts. Inclusion complex formation between the CD hosts and the aromatic guests in nonpolar solvents were evaluated by NMR spectroscopy. The association constants between the CD hosts and the aromatic guests were determined by ¹H-NMR titration method. These CDs effectively formed 2:1 inclusion complexes with pyrene in benzene-d⁶ and cyclohexane-d₁₂ with considerably high association constants. The 2:1 inclusion complex formation between TIPS-beta-CD and pyrene in nonpolar solvents was also confirmed by the APPI-MS analysis. Crystallographic study of the TIPS-beta-CD/pyrene inclusion complex obtained from the benzene solution showed the formation of a unique ternary complex among TIPS-beta-CD, pyrene, and the solvent. Interestingly, the pyrene molecule forms a sandwich-type complex with two benzene molecules through pi-pi interactions, and is located at the center of the dimer cavity. The crystalline structure suggested that the interaction between the solvent and pyrene affects the mode of pyrene inclusion within the CD dimer cavity.

Novel aspects:
This is the first report on the structural analysis of cyclodextrin inclusion complexes formed in nonpolar media.
A NEW CHEMOMETRIC APPROACH FOR THE QUANTITATION OF STEROIDAL COMPOUNDS IN WASTEWATER BY GCxGC-TOFMS

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Keywords: GCxGC-TOFMS, chemometrics, emerging organic contaminants, steroids, wastewater

An increasing variety of chemical compounds including pharmaceuticals, personal beauty products and insect repellents are offered daily to consumers. The final destination for most of these compounds is the environment as raw compounds and their metabolites, some of which are potentially hazardous for the environment. In order to speed up the process of regulation and assessment of environmental threats, it is essential to develop rapid and efficient methods for the analysis of these contaminants.

In this study, exhaustive sample preparation method was first developed for the determination of steroidal species in the wastewater samples aiming at the clarification of the distribution of free and conjugated steroids in wastewater and suspended particles. Special emphasis was put on the applicability of comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS) to screen steroidal contaminants in wastewater. Although the resolution and sensitivity of GCxGC-TOFMS is superior over those of conventional GC-MS techniques, the lack of available commercial standards can hinder the usefulness of the technique, especially if the goal of the analysis is the quantitation of the identified compounds. Several approaches based on the use of surrogates can be found in the literature for the quantitation of compounds identified by MS, but frequently they lack the accuracy. In this study a new approach based on partial-least-squares regression (PLSR) was developed for the quantitation of emerging steroidal compounds in water and sludge samples taken from the largest wastewater treatment plant of Finland.

Six commercial steroidal compounds injected to the GCxGC-TOFMS at seven concentration levels ranging from 0.1 to 10 ng, were used as reference compounds for the development of the PLSR equation, which was successfully exploited for the determination of more than 40 steroidal compounds present in the analyzed samples.

Novel aspects:
The quantitation of unknown emerging organic contaminants with a chemometric model based on partial least squares regression equations, which were built up using six commercial steroid standards.
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-contaminants, 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.
Hydroxy polychlorinated biphenyl (OH-PCB) is one of the intravital metabolites of polychlorinated biphenyl (PCB). Many cases about measurement OH-PCB in biological samples were reported and they pointed out that some of the isomer has an effect on thyroid hormone action or female hormone action. In the case of environmental samples Sakiyama et al. (2007) indicated the presence of OH-PCB in an aqueous environment and bottom sediments. The result suggests that PCB is also hydroxylated in environment by physicochemical reaction or metabolic reaction of living organisms.

At our previous research (2008) on the actual situation of OH-PCB in soil there are correlations between the OH-PCB concentrations and the PCB concentrations regardless of whether the soil is contaminated. We also reported that OH-PCBs are formed from a mixture of PCBs (KCmix) under various conditions. No report is available that actually researched about the formation mechanism of OH-PCBs from dominant PCB congeners. In order to confirm the mechanism of OH-PCB formation, we selected 10 dominant congeners of PCB (#3, #12, #26, #31, #52, #101, #118, #153, #180, #209) as parent to hydroxylate under two conditions as follows. The first was irradiating UV-light to the silica gels, and the second was keeping the soils under light-shielding with addition individual PCB congeners. The OH-PCBs formed in the test samples were pretreatment through several steps and then methylated for analysis. The methoxy-PCBs were detected based on mass spectra characteristics after gas chromatography separation. In addition, they were identified by comparing their retention characteristics with methoxy-PCBs those were methylated from the available OH-PCB standards provided by reagent manufacturer (47 congeners) and Okumura synthesized (80 congeners). We present the new findings about congener specific formation of OH-PCB that will be the help for the following studies on OH-PCB in environmental samples.

Reference literature
1) 16th Symposium on Environmental Chemistry, Sakiyama et al., 408-409 (2007)
2) 17th Symposium on Environmental Chemistry, Shimase et al., 480-481 (2008)

Novel aspects:
Congener specific formation of OH-PCB with UV-light in silica gel or without light in soil
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 the individual compounds based on a 1 mL injection. For confirmation of each compound, the exact mass of the compound, its isotope pattern and as well as the MS/MS spectrum produced were collected and compared against a fragmentation library which prevented “false positives” in the results. One of the main challenges using a high resolution accurate mass system is data mining. In this work we will show the data to screen, quantify and confirm in a single run therefore, spending less time on the instrument for repeat runs. Furthermore, the online pre-concentration step allows for low detection limits of these compounds without the time consuming steps of offline solid phase extraction of 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
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.
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 microorganisms. 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 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
Analysis of thermal products of chlorpyrifos using LC/FTMS

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

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-trichloropyridinol (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 5µm 2.1 x 250mm (GL Science). The best LC separation for the thermal 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, trimer and tetramer ions increased as the peak intensity of 3,5,6-TCP decreased in the mass spectra. The analysis of separation behavior for the LC-MS chromatogram indicated that these polymeric species contained some structural isomers. The new finding obtained from the present LC/FTMS measurements is that the heating of chlorpyrifos generates its oligomers by polymerization. The oligomers are shown to have a wide variety of isomers with different binding sites of chlorine atoms.

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.

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Behavior of Hexabromocyclododecane (HBCD) stereoisomers in water, sediment, and biological samples.

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Keywords: brominated flame retardants, 1,2,5,6,9,10-hexabromocyclododecane, LC/MS/MS, environment

1,2,5,6,9,10-hexabromocyclododecane stereoisomers (HBCDs) are used as flame retardants in thermal insulation building materials, upholstery textiles, and electronics. Since 2004, the production and use of pentamix- and octamix-polybrominated diphenyl ethers (PBDEs) have been restricted in Europe. HBCDs might be used as an alternative for PBDEs, and are the second highest-volume brominated flame retardants (BFRs) used worldwide, after tetrabromobisphenol A. Because of the widespread use and the physical and chemical stability, HBCDs are now ubiquitous contaminants in the environment. In Nagoya city, HBCDs were frequently measured in environment. It is required to reveal their environmental distribution, behavior and time trends.

Here we report the concentrations of HBCDs in water, sediment, and biological samples, and analyze the biomagnification potential, the stereoisomer profiles, and the time trends. Analysis was undertaken by liquid chromatography/ tandem mass spectrometry (LC/MS/MS) on a diastereoisomer basis. Ninety-three samples (22 of river and sea water samples, 27 of sediment samples, 24 of fish and shellfish samples, and 20 of breast milk samples) in Nagoya city were measured.

HBCDs were detected in 18 water samples, of which concentrations of total isomers ranged from 0.4 to 280 ng/L. γ-HBCD dominated over the other isomers in water samples. Similarly, sediment samples were dominated by δ-HBCD, which was detected in all samples, ranged from 0.2 to 27 ng/g-dry. Time trend analysis by boring sediment core samples in Nagoya port showed a remarkable increase in HBCD concentrations since about 2000. This might be reflected by changing use of HBCDs from PBDEs according to regulation of EU on the production and use of two PBDEs. In fish and shellfish samples, the maximum HBCDs concentration was about 5-10 times that reported in earlier Japanese studies. δ-HBCD was the predominant isomer of HBCDs and was found in all the samples in levels from 24 to 6300 ng/g-lipid. On the other hand, γ-HBCD was predominant in some samples, and the isomer patterns of HBCDs were similar to those in industrial products. Samples in which γ-HBCD concentrations were higher might be collected near the source.

In breast milk samples, δ-HBCD was also predominant in HBCDs and was found in all samples in concentrations from 1.5 to 13 ng/g-lipid. The concentrations of HBCDs in the samples of the same person decreased from 7 to 1.5 ng/g-lipid during 9 days to 300 days of post parturition.

Novel aspects:
HBCDs regional contaminations in Nagoya city were revealed by measuring river waters, sea waters, sediments, fishes and breast milks.
LC/MS/MS determination of hair-dye ingredients in products, water and urine: estimation of human exposure and environmental release.

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Keywords: exposure, environment, release, hair-dye

Introduction: Over 5,000 different chemicals are used in hair-dye products, some of which are reported to be carcinogenic and/or allergic. Aminophenol, diaminotoluene, aminocresol and resorcinol are well used and hazardous ingredients of hair-dye. The authors developed an LC/MS/MS method for simultaneously determining the hair-dye ingredients and applied it to the estimation of human exposure and environmental release of the ingredients.

Methods:
(1) measurement of the chemicals in shampoo and rinse water
After coloring, the hair was shampooed with one push (16g) of shampoo bottle. Then the hair was rinsed with water (5L), which was collected in a plastic bucket. A hundred milliliter of the rinsed water was loaded to Solid Phase Extraction Cartridges (C18 and Oasis MCX plus were combined in series). The elute with methanol (5ml) from C18 and the elutes with methanol (5ml), ammonia/methanol solution (1/19, 5ml) from Oasis MCX plus were separately concentrated to near 0.5ml under nitrogen gas stream. The concentrates were diluted with acetonitrile, filtrated and prepared in 2ml for LC/MS/MS analysis.

(2) measurement of the chemicals in urine.
Within 24 hours after coloring the hair, all the urine were separately collected in glass bottles and stored in cool box. Every ten milliliter of urine was diluted with 40ml of water, and applied to the SPE. Samples were prepared with similar way to above.

Predominant result:
(1) determination of hair-dye ingredients in shampoo and rinse water
For the SPE method development, five kinds of SPE cartridges were examined, i.e. Slim-J PRS, AC-2, NEXUS, HLB and MCX. Although good recovery was not obtained with any of them, MCX had a potential to collect hydrophilic hair-dye ingredients by connecting it with other SPE cartridge. It was the best in the recoveries to connect C18 cartridge upstream of MCX. With the series connection of C18 and MCX, the recoveries of 5-amino-o-cresol, 2,5-diamino-toluene, p-amino-phenol and resorcinol spiked in shampoo and rinse water had improved from 15.5\% to 96\%, from 39\% to 76\%, from 21\% to 56\%, and 0\% to 28\%, respectively. An effect of pH on recovery was examined, because the MCX performs the best under pH 3~4. But it was concluded that higher recovery efficiency was obtained under pH value of 9.5 than that of 3.

A shampoo and rinse water sample was analyzed at 0, 2, 4, 8 and 24 hours afterward. The examined hair-dyes were two kinds, i.e. black and golden. The ingredients of the dyes differ to each other; 2,5-diamino-toluene and p-amino-phenol are contained in the black dye, and 5-amino-o-cresol, p-amino-phenol and resorcinol are in the golden. With progress of time, the concentration of every substance decreased. In particular p-amino-phenol which was contained in golden hair-dye showed remarkable decrease in concentration from 4 hours after the shampoo. And as for resorcinol, the rate of the decrease was much slower than that of others. Further study is required to pursue the persistence of resorcinol.

(2) determination of hair-dye ingredients in urine
It is still under examination. Because of many impurities in urine, more efficient sample preparation method is required.

Novel aspects:
Human exposure and environmental release of hair-dye ingredients were examined by developing measurement method by LC/MS/MS.
Qualitative analysis of waste leachate by using exact mass of LC/Q-ToFMS/MS

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Keywords: LC/Q-ToFMS/MS, MetFrag, irregular dumping

Introduction:
Environmental pollution by chemical substances has been a serious problem. In our daily life the various types of chemical substances are used and wasted. The authors aim to develop a method comprehensively separating chemicals according to their properties and qualifying them with LC/Q-ToFMS/MS. Here we report a qualitative analysis of a leachate from irregular waste dumping area by a provisional qualification method with LC/Q-ToFMS/MS.

Methods:
To separate a variety of chemicals, a solid phase extraction (SPE) method using C18 cartridge followed with ion exchange cartridges (Oasis MAX, WAX, MCX, WCX) was developed. Chemicals collected in the SPE cartridges were fractionated with the way as follows; Two liter of water sample was loaded into C-18. Substances collected in C18 were eluted with 10mL of methanol. The water passed through C18 was divided into four equal parts of 2L. The every 500mL of C18 passed fraction was separately loaded into Oasis MCX, MAX, WAX and WAX. From the MCX and the WAX, collected substances were eluted with 10mL of methanol/ammonium solution. From the MAX and the WCX, collected chemicals were eluted with 10mL of methanol/formic acid solution. All the fractions were concentrated to 2mL in volume and served for accurate mass measurement using LC/Q-ToFMS/MS.
The exact mass spectra were analyzed with ‘MetFrag’ (Leibniz-Institut für Pflanzenbiochemie) by ways as follows; the elemental compositions of intact molecular ions were analyzed with the mass accuracy of better than 3 ppm. Then molecular structures that agree with the elemental composition were extracted from ‘ChemSpider’ (chemical structure database by UK in the 26 million structures) and the structures of which predicted fragments match the sample spectrum (with mass accuracy of better than 6 ppm) were chosen. Chemicals in a leachate from irregularly dumped waste were qualified by the method written above.

Predominant result:
Hundreds or more candidates of chemicals were extracted by simply eliminating chemicals of which intact molecular ions didn’t fit the mass spectra with the mass accuracy of better than 3 ppm. It could be possible to identify some chemicals from the sample mass spectra with the combination of the product ion analysis with mass accuracy of better than 6ppm. In a leachate from irregular waste disposal site, pharmaceuticals, flame retardants, surfactants and other chemicals were found; VALGANCICLOVIR (0.28 ppm Antiviral agents), Eto- droizin dimaleate (1.91 ppm Sedative), Phosphoric acid-tris-(2-butoxyethyl) (0.75 ppm), Dimethylaminophenol (2.19 ppm Resin hardener) and a polyethoxylate [-OCH₂CH₂-] surfactant were found. The LogP values of the substances above were ranged from-1.27 to 3.50. The phosphorus-based flame retardant would be from industrial waste. The pharmaceuticals might be from a different source from industrial wastes. The presented method is provisional until it has been validated with various environmental samples.

With the presented SPE method, hydrophilic and hydrophobic substances could be simultaneously collected, fractionated and qualified by LC/Q-ToFMS/MS.

Novel aspects:
The simultaneously fractionation method followed by LC/ToFMS/MS was developed. The exact masses of product ions and precursor ions were analyzed by using MetFrag.
Investigation of Perfluorinated compounds in Osaka-bay over past three years

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Keywords: Perfluorinated compounds, Osaka-bay, Environmental investigation, LC-MS/MS

Perfluorinated compounds (PFCs) have been widely used by various consumers and industrial products for over 50 years. PFCs are used for various products commencing with surface protectors in carpets, paper, packaging, and fire fighting foams. However, PFCs, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been found to be persistent, bioaccumulative, and toxicant. Furthermore, many studies have been reported on global distribution of PFOS, PFOA and the homologues, such as perfluorinated carboxylic acids (PFCAs) and perfluorinated alkyl sulfonates (PFASs). PFCs have been detected in human bloods, biota, sea water, and remote areas such as the Arctic.

In Japan, PFCs contamination, especially PFOA, in Keihanshin area (the area over Osaka prefecture, Kyoto prefecture, and Hyogo prefecture) were recognized by many studies. On the other hand, fluoro resin manufacturers and fluoro resin suppliers are reducing emissions and product content of PFOA, precursors, and higher homologues based on the USEPA's "2010/15 PFOA Stewardship Program". Some companies are using PFCs of C6 such as Perfluorohexanoic acid (PFHxA) as alternative materials. Composition of PFCs in the environment may change reflecting these activities. In addition, serum half-life in rats and monkey and some toxic properties of PFHxA was lower than PFOA. Therefore, it is considered that risk of PFHxA is smaller than PFOA.

In this study, to confirm the concentration and composition of PFCs in the coastal region of Osaka-bay, Japan, total of 12 PFCs, which were PFOA, PFOS and the homologues, were investigated. The coastal region of Osaka-bay is one of the main densely-populated and industrial areas in Japan. It seems that the concentrations of PFCs in the sea reflect emission from points and/or non-point source of the coastal land area. The sea water samples were collected in 2008, 2009, and 2010. The samples were stored in 4°C until analysis. The sample was extracted by a solid phase extraction cartridge. The loaded cartridge was eluted by Methanol. The eluted solution was concentrated to 1mL. The final solution was carried out by using liquid chromatography (LC)-tandem mass spectrometer (MS/MS). The analysis was conducted using negative electrospray ionization with multiple reaction monitoring.

PFHxA and PFOA were detected in all samples. Concentration of PFHxA ranged from 11 to 760 ng/L. Concentration of PFOA ranged from <MQL to 62 ng/L. Composition ratio of PFHxA was dominant in all samples. The concentrations in the coast had been reported few ng/L or few hundred ng/L by other studies. The concentrations of PFHxA and PFOA in this study were of similar value to other literatures. However, the concentration of PFCs in pacific, which had been considered that there is no influence from defined source, had been reported few pg/L or few ten pg/L. It seems that there is discharge of PFCs from the coast to the sea. Additionally, as for the characteristics of spatial distribution of PFCs, concentrations at closed-off section were high. PFCs might be discharged from closed-off section. Concentrations of PFHxA were increased by one order of magnitude from 2008 to 2009. It seems that amount of the discharge had mounted in around 2008.

Novel aspects:
Contamination of PFCs in Osaka-bay has been insufficient information. This research presented the characteristic distribution and the change of concentrations of PFCs in Osaka-bay.
Computational Chemistry Study on Negative Ion Chemical Ionization Mechanism of Peroxyacetyl Nitrate

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Keywords: Electron capture chemical ionization, DFT, MOPAC, Photochemical oxidant

Atmospheric photochemical reactions of non-methane hydrocarbons (NMHCs) and nitrogen oxides (NOₓ) produce wide variety of peroxyacyl nitrates (PANs, RC(O)OONO₂). They transport NOₓ far downwind to affect the photochemistry in remote areas. PANs affect adversely on human, and their mutagenic activities are species-dependent. Recently, negative ion chemical ionization/mass spectrometer (NICI/MS) was reported as a sensitive and selective detector for PANs. On the other hand, their explosive and thermally unstable nature makes it difficult to synthesize their authentic standards. In this study, the behaviors of peroxyacetyl nitrate (PAN, CH₃C(O)OONO₂), the most common class of PANs, in the NICI/MS source was investigated by means of computational chemistry study to fully understand the production mechanism of the dominant ions by means of density functional theory and semi-empirical molecular orbital calculations.

The NICI process was initiated by the electron attachment on PAN. The resulting PNA⁻ seemed highly unstable with respect to the O-O bond dissociation. Besides, the O-N bond seemed also dissociable for PAN⁻ at the Frank-Condon state. The possible neutral products from the two dissociation channels were electrophilic, and therefore could capture additional electrons to ionize in the NICI/MS source. Accordingly, NO₃⁻, NO₂⁻, CH₃C(O)O⁻, and CH₃C(O)OO⁻ were possible product ions during the NICI/MS analysis of PAN.

Novel aspects:
The fragmentation and ionization mechanisms of PAN was theoretically elucidated. This helps us to guess the major NICI/MS fragment ions of unidentified PANs.
Analysis of inadvertent PCBs contained in consumer goods

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Keywords: inadvertent PCB congener yellow pigment

Although production and use of polychlorinated biphenyls (PCBs) were banned in early 70s in Japan, safe storage and disposal of PCBs are still social concern. In addition to that, “irregular” PCB congeners, showing different composition from PCB products, were found unexpectedly in wastewater (1) followed by in air and river water (2, 3), giving rise to another social and technical concern. The origin of such “irregular” congeners has been studied and elucidated (2); they are from polychlorobenzidine dyes and production process thereof and released to the environment finally.

Among 209 PCB congeners, 3,3’-dichlorobiphenyl (PCB 11) and 2,5,2’,5’-tetrachlorobiphenyl (PCB 52) are the most typical inadvertent ones. These congeners in some organic pigments showed different composition profile from the one seen in ordinary PCB products. This means that some portion of PCBs found in the environment may be the byproducts of pigment. Little attention had been paid for them until recently, though US Code of Federal Regulations pointed out the unintentional formation of such byproducts in the yellow pigments in 1979 (4).

Based on such a background, Japanese Government placed an official request for emergency survey of PCB content as for several certain pigments against manufacturers and importers thereof in February 2012 (5). Several consecutive actions have been undertaken so far.

From a point of risk management, it is more important to know the exact congener composition than to know total PCB content, as every congener has its own toxicity varying from the top to the bottom. For such a precise purpose, powerful high resolution GC-MS is preferably used instead of conventional GC-ECD, because GC-ECD, often used for PCB determination in electrical condenser oil, cannot differentiate congener peaks.

Organic pigments have replaced traditional Cadmium Yellow aiming to avoid cadmium, and they are now used for wide variety of industrial products as well as daily consumer goods. It is quite rational to think “Do yellow retail products carry such congeners?” Some analytical results are available in the literature (6), however ordinary consumers may know very little about the material composition of consumer goods. That is why upstream compound maker may have it closed with secrecy reason. Therefore we are somehow anxious about yellow stuff, whether it contains unintentional PCB congeners or not.

Analyses of inadvertent PCBs contained in yellow consumer goods such as crayon, ink, paint, lacquer, adhesive tape and so on were carried out by utilizing capillary GC equipped with high resolution MS in this study. In comparison to the environmental samples such as air and water, sampling itself was easy as for consumer goods. However, it seemed to be difficult to construct typical one standard method so as to cover all samples we dealt. From an experimental viewpoint, clean-up protocol shall be modified case by case according to the property of test sample.

Analytical results obtained are to be presented in this paper.

References

Novel aspects:
Some PCB congeners showing irregular composition profile were found in some pigments, and becoming social concern. Analyses of inadvertent PCBs contained in consumer goods were carried out.
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 retained in human and wildlife blood. Among those compounds, a large number of hydroxylated polychlorinated biphenyls (OH-PCBs) have earlier been reported in blood from humans, terrestrial and marine mammals.

Some analytical methods, which employed mainly gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) using electron ionization mode (EI) have already been applied for OH-PCBs analysis. Although GC-HRMS(EI) can provide high sensitive and selective data, the instruments are extremely expensive for routine and high throughput 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 into neutral (containing PCBs) and phenolic (containing OH-PCBs) fractions with KOH(1M ethanol/H$_2$O, 1:1, v/v). The alkaline phase containing OH-PCBs was acidified to pH 2 with sulfuric acid and re-extracted with MTBE/hexane. OH-PCBs fraction was passed through non-activated silica-gel column chromatography. OH-PCBs were derivatized with trimethylsilyldiazomethane. The derivatized solution was further cleaned up by using activated silica-gel column. Identification and quantification of MeO-PCBs were analyzed by GC-LRMS (ECNI) and compared to GC-HRMS (EI).

Firstly, we compared the instrumental detection limit (IDL) of OH-PCBs in GC-LRMS (ECNI) and GC-HRMS (EI). The IDL was defined as 3 times the standard deviation (SD) of 5 replicate injections of a low concentration standard solution of OH-PCBs (1 pg ml$^{-1}$). The IDLs were 4-OH-triCB29 (22 pg), 4-OH-tetraCB79 (14 pg), 4-OH-pentaCB107 (0.65 pg), 4-OH-hexaCB116 (1.6pg), 4OH-hexaCB159 (0.90 pg), 4-OH-hepta-CB172 (0.81 pg),4OH-heptaCB187 (0.57 pg) and 40H-octaCB201 (0.63 pg) by GC-ECNI-MS analysis, and compared to 4-OH-triCB29 (0.82 pg), 4-OH-tetraCB79 (0.56 pg), 4-OH-pentaCB107 (0.34 pg), 4OH-hexaCB148 (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(EJNI) was in agreement with the GC-HRMS (EI) method. However, sensitivities of GC-LRMS (ECNI) to tri- to tetra- OH-PCBs of GC-LRMS(EJNI) 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).
Suspended solid as a disturbance of PFOS analysis in case of wastewater

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Keywords: PFOS SS surrogate recovery COP4

Perfluorooctane Sulfonate (PFOS) is one of the Perfluoroalkane Sulfonates (PFASs) and is also the terminal degradation product of many perfluoroalkyl compounds (PFCs). Specific properties of PFCs such as water repellency, thermal stability, and surface tension lowering, made these compounds important both for commercial and technical aspects.

As a consequence, PFOS having several emission paths to the environment had been broadly spread out over 50 years. Recently PFOS was designated and restricted as persistent organic compounds (POPs) by the Stockholm Convention on POPs (1).

In Japan, PFOS was specified to the first class chemical under the Chemical Substance Control Law followed by filing technical guideline for disposal of PFOS containing garbage in March 2011 (2). Several kinds of standard analytical method using LC-MS/MS (2-4) had been proposed for surface water prior to that.

Many papers pointed out the technical difficulties arising from the contamination owing to fluorine resin used for connectors or tubing material in the apparatus, even when clean water was analyzed (5).

Other than contamination, we encountered different difficulties when we applied current analytical methods to the wastewater in the beginning of our study. We experienced low recovery of surrogate substance that was added to the sample to ensure the precision. Low surrogate recovery did not meet the requirements prescribed in the current analytical standards designed for surface water.

We found the reason for the difficulties. On one hand, it is known among the lab analysts that concentration of PFOS is prone to be decreased probably owing to the adsorption toward labware. But on the other hand, little attention was paid for the adsorption onto suspended solid (SS) in case of surface water, in spite of numerous efforts to develop sampling and analytical methods for PFOS, since SS content in surface water is generally enough low (6).

Development of harmless disposal method undergoes, analysis of wastewater we concern shall be required. Proper care should be taken for adsorption on SS for the analysis of water containing high SS.

Among elution conditions of PFOS, eluent composition was intensively investigated, and in addition we found both quality and quantity of SS affected on surrogate recovery, leading to an improvement toward current method. We would suggest the importance of SS remained on the filter upon analytical results.

The last portion of this study relates to the effect of perfluorinated chain length on the recovery, and results are to be discussed.

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References

Novel aspects:
Recovery of surrogate substance in PFOS analysis was investigated to improve current method, and the effect of chain length of perfluorinatedalkane on the recovery is to be discussed.
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 Spectrometry; 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

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.
Measurement of brominated flame retardants in the environment near factories by LC/MS/MS.

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Keywords: BFRs, road-dusts, LC/MS/MS, factories

Introduction: Brominated flame retardants (BFRs) are widely used in plastics, rubbers, textiles and other products for fire protection, and are discharged into the environment through air emissions, water discharges, wastes and end products. In the previous studies, the authors found unexpected contaminations with BFRs in not only plastic products such as baby toys, chew toys and household products, but in road dust around the non BFR plastic moulding factories. Here we present a research predominantly taken place around factories by measuring BFRs in effluents, river waters, sediments and road dusts.

Method: Waters, sediments and road dusts were collected in rivers, effluent water ways or road sides near the factories. Water samples were added with formic acid (500 µL to every 500mL of water) and stored in cool and dark place. Sediment samples were collected in glass bottles and stored in freezer. Road dusts were sampled in glass bottles and stored in cool and dark place. Water sample was filtered and the filtrate was extracted by solid phase extraction (SPE) with C18 sorbent (Inert Sep C18, 500 mg) which was conditioned with methanol (5mL) and distilled water (5mL) in the order. The sample loaded cartridge was cleaned by flushing with 50%methanol (1mL), and then the target substances ware eluted from the cartridge with methanol (5mL) and acetone (5mL). The residual suspended solids on the filter and the glassware used for sample preparations were rinsed in the order with acetone (5mL) and dichloromethane (5mL), which were added to the C18 extract. The extract was concentrated with nitrogen, and the solvents were exchanged to acetonitrile followed by LC/MS/MS (with API3000). Sediments and road dusts were extracted in the order with acetone (5mL, two times) and dichloromethane (5mL, two times). The extract were concentrated and cleaned up by SPE with C18, and measured by LC/MS/MS.

Predominant result: The method recoveries were studied by analyzing five replicates of samples spiked with Hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), tribromophenol (TBP) and bisphenol A (BPA). Recoveries of HBCD, TBBPA, TBP and BPA were 63% with relative standard deviation (RSD) of 8%, 102% with RSD of 17%, 98% with RSD of 12% and 106% with RSD of 4%, respectively. It would be the reason for the lower recovery of HBCD (around 60%) that the HBCD is extremely hydrophobic substance, which might be adsorbed on the surface of glassware, SPE adsorbents and other hydrophobic materials.

In research of a dyeing processing factory, water concentrations of TBP and BPA were 1.3-6.3ppt and 9.0-950ppt, respectively. On the other hand, those of HBCD were lower than limit of detection in most samples excepting in the samples of the effluent and near the effluent discharge point in a river. All other results will be presented in the poster.

Novel aspects:
BFRs in river waters, sediments, effluents and road-dusts near factories were determined by LC/MS/MS.
Determination of sulfonamides and tetracyclines in livestock wastewater using hybrid ion trap - time of flight mass spectrometer

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Keywords: Sulfonamide, Tetracycline, Ion Trap, Time of Flight, Wastewater

Veterinary drugs are widely used in large quantities for treatment of disease or to promote animal growth. Nowadays, veterinary drugs are recognized as not only medicines but also as 'Newly Emerging Contaminants' in the environment. The aim of this study is to monitor organic compounds in aqueous samples using Liquid chromatograph coupled to hybrid ion trap - time of flight mass spectrometer. The analyzed compounds were 3 sulfonamides (i.e., sulfathiazole, sulfamethazine, and sulfamethoxazole), and 2 tetracyclines (i.e., oxytetracycline and chlortetracycline) in samples from a livestock wastewater treatment plant (WWTP). Sample extraction was carried out with a hydrophilic-lipophilic balance and a mixed-mode cation exchange solid phase extraction cartridges. Average recoveries of sulfonamides at fortification levels from 1.0 and 4.0 μg L⁻¹ in effluent of a local domestic WWTP were 73-95% and 89-104%, respectively, while tetracyclines at 0.4 and 4.0 μg L⁻¹ were 76-104% and 101-107%, respectively. The method detection limits were 22.8, 23.0, 25.8, 23.6, and 9.8 ng L⁻¹ for sulfathiazole, sulfamethazine, sulfamethoxazole, oxytetracycline, and chlortetracycline, respectively. The maximum concentration of sulfonamide and tetracycline residues detected in samples were 49.5 and 4.1 μg L⁻¹, respectively. The developed method could be applied successfully to quantitate residual sulfonamides and tetracyclines in animal wastewater. The analysis of the samples showed that more than 90% of target pharmaceuticals were removed in the animal WWTP consisting of a biological process, a UF membrane, and a coagulation process.

Novel aspects:
Analytical method of sulfonamides and tetracyclines using hybrid ion trap - time of flight MS was described. Sample extraction was carried out with a HLB and a MCX cartridges.
Dechlorane Plus, a highly chlorinated flame retardant in Japanese environment samples.

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Keywords: Dechlorane Plus, Japan, Environment

Dechlorane Plus (DP) is a highly chlorinated flame retardant, was developed to replace Mirex (Dechlorane) that was banned in the 1970s. It is manufactured about 450 ton per year by Occidental Chemical Corporation (OxyChem) in the United States. In 2006, the first of sightings of this chlorinated flame retardant were in the Great Lakes region, and the investigation reports are increasing rapidly recently in the world, mainly in North America. More recently, a newly DP production facility was discovered in China. It is estimated that Chinese capacity of DP production has reached 2,000 ton in 2006.

However, to our knowledge, no data for levels of DP in environmental samples were available in Japan. Therefore, we measured DP by using the GC / high resolution MS in EI mode, and identified this compound in the environmental samples collected from Japanese urban area. DP was detected in house dust, deposit of the window frame, road sediment, garden soil and sediment samples at concentrations ranging from 2.9-42 ng/g-dry, 240-270 ng/g-dry, 74-150 ng/g-dry, 1.7 ng/g-dry, 17-140 ng/g-dry, respectively. Further, we showed the mean of anti-DP fractional abundance ($f_{anti}$) value in these Japanese samples was 0.65, 0.83, 0.80, 0.81, 0.81, respectively. This compound had not been identified in the environment of Japan before our report. In this conference, we will indicate the detailed additional data of DP in surface sediment samples of the urban river in Japan.

Novel aspects:
Dechlorane Plus was determined in environment samples for the first time in Japan.
Multi-residue method for rapid screening of veterinary drugs in muscle matrices by UHPLC-MS/MS

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Keywords: Multi-residue screening, Veterinary drugs, UHPLC-MS/MS

A rapid multi-residue screening method was developed for the simultaneous analysis of about 180 veterinary drugs and their metabolites using a ultra-high-performance liquid chromatograph coupled with tandem mass spectrometer (UHPLC-MS/MS). The screened veterinary drugs belonged to amphenicols, anthelmintics, benzimidazoles, β-lactams, coccidiostats, ionophores, macrolides, non-steroidal anti-inflammatory agents, quinolones, sulfonamides, tetracyclines and tranquilizers. The drugs were extracted from bovine, porcine, and chicken muscle samples with acetonitrilewater (4:1, v/v) containing 2 mM ammonium formate, and the extracts were applied to the dispersive solid phase extraction and n-hexane clean-up procedure. Reverse-phase LC separation was accomplished on a C₁₈ column and gradient elution with a mobile phase consisting of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The method had a chromatographic total run time of 5 min. The separated compounds were detected with a Shimadzu LCMS-8030 tandem quadrupole mass spectrometer operating with an electrospray ion source (ESI) in positive and negative switching mode by applying a time scheduled multiple reaction monitoring of 2 or 3 transitions. The developed method was validated according to the EU Commission Decision 2002/657/EEC for a quantitative screening method. All the validation data, such as the mean accuracy, the repeatability, the within-laboratory reproducibility and the detection capability, CCβ accuracy were within the required limits.

Novel aspects:
A rapid multi-residue screening method was developed for the 180 veterinary drugs and their metabolites using a UHPLC-MS/MS.
Development of LC-MS/MS method to monitor pharmaceuticals in environmental wastewater

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Keywords: Pharmaceutical, Wastewater, UHPLC-MS/MS

A rapid and reliable LC-MS/MS method was developed for the simultaneous identification, confirmation and quantification of 30 pharmaceuticals in water and wastewater samples. Two distinct chromatographic conditions were used according to the polarity. The method used sub two micron size C18 and hydrophilic interaction chromatography columns. The separated compounds were detected with a Shimadzu LCMS-8030 tandem quadrupole mass spectrometer operating with an electrospray ion source (ESI) in positive and negative switching mode by applying a time scheduled multiple reaction monitoring of 2 or 3 transitions. The analytical performance of the method was evaluated for sample collected from a local domestic wastewater treatment plant. Sample extraction was carried out with a hydrophilic-lipophilic balance and a mixed-mode cation exchange solid phase extraction cartridges. To minimize the matrix effect, matrix-matched standards were analyzed in a wastewater effluent. Under the study, all compounds gave good sensitivity over a level of ng L$^{-1}$ with $r^2$ values of 0.99 or greater. Average recoveries of all compounds at 10 and 1,000 ng L$^{-1}$ were about 54-105%.

Novel aspects:
An analytical method for screening and confirming of 30 pharmaceuticals in water and wastewater samples was developed using a UHPLC-MS/MS.
Analysis of Ultraviolet Absorbers in Urine Samples by Functionalized Nanomaterial-assisted Electrospray Mass Spectrometry

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Keywords: Ultraviolet Absorbers; Functionalized Nanomaterial-assisted Electrospray Mass Spectrometry

Functionalized nanomaterials assisted electrospray ionization for an analysis of ultraviolet absorbers in urine was developed in this study. The electrospray was performed with high voltage on a laboratory-made screen-printed plate with coating a layer of silver or graphite. The ultraviolet absorbers studies in this study are including 3-(4-methylbenzylidene)-camphor (4-MBC), 2-ethyl-4-(dimethylamino)benzoate (OD-PABA) and 2-hydroxy-4-(octyloxy)-benzophenone (BP-12). The analytes were extracted with nanomaterials that included iron oxide-coated silicon dioxide nanoparticles, multiwall carbon nanotubes (MWCNTs) and graphene oxide (GOx) was dipped on the screen-printed substrate to evaluate the effect on the ionization efficiency. After extraction, the extract or nanomaterial without doing any desorption. From the results, the MWCNTs can enhance the intensity of the signal detected and short the analytical time. This technique demonstrates that the nanoparticles assisted electrospray ionization offers a high specific and fast screening in trace analysis.

Novel aspects:
The nanomaterial-assisted electrospray ionization offers a high specific and high throughput screening for trace analysis.
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.
New Perspectives in the Mass Spectroscopy Determination of Dioxin-like Substances in Environmental and Food Samples

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Keywords: dioxin-like substances, triple-quadrupole, GC-QqQ(MS/MS), HRGC-HRMS

Due to an increased concern of the dietary exposure of dioxin-like compounds to the general public, the accurate determination of these compounds has become of great interest. These compounds are found in the environment and food matrices at very low levels therefore analytical techniques and instrumentation providing precision, sensitivity and adequate limits of detection are necessary.

In the past dioxin-like compounds have been analyzed with gas chromatography coupled to single quadrupole and ion trap mass spectrometry, two-dimensional gas chromatography coupled to micro electron capture detection, and time-of-flight mass spectrometry. These methods are alternatives to the current reference instrumentation of high resolution gas chromatography high resolution mass spectrometry (HRGC-HRMS) for the analysis of many persistent organic pollutants, mainly dioxin-like compounds. In general, dioxins are analyzed at 10,000 resolution, ionized with a positive electron impact (EI+) source and quantified based on the isotope-dilution method. Magnetic sector remains the choice instrument for the analysis of dioxins as it allows for the necessary sensitivity at very low levels and the high resolution to separate the peaks. Although HRGC-HRMS is commonly used to analyze for dioxin-like compounds, this instrumentation is costly and high maintenance therefore alternative instrumentation are continuously under development. Gas chromatography coupled to tandem mass spectrometry with a triple quadrupole analyzer (GC-QqQ(MS/MS)) seems to potentially have the sensitivity and selectivity compared to HRGC-HRMS. Transitions from different precursors to product ions have been evaluated at several collision energies in order to determine the optimal conditions for the analysis of PCDD/Fs and DL-PCBs by GC-QqQ(MS/MS). Results are compared to those obtained by GC-HRMS in order to assess the applicability of this technique for the analysis of dioxins in environmental and food samples.

Comparative analyses were performed on a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) equipped with a triple quadrupole (TSQ Quantum XLS, Thermo Fisher Scientific, Bremen, Germany) and on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a DFS high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) controlled by an Xcalibur data system.

For optimization of the multiple reaction monitoring (MRM) method, different transitions were studied in order to select the most intense and, if possible, to achieve the highest number of identification points. For PCDD/Fs, the loss of the COCl group was the most abundant when collision induced dissociation (CID) voltages around 25 and 30 V were applied. Collision energies were always higher for PCDFs. In addition, other transitions were observed and studied when CID was set to higher values (40-50 V). Similar behaviour was observed for DL-PCBs, with the most intense transitions from the molecular cluster to the loss of two chlorine atoms for the congeners investigated.

Acknowledgements

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References


Novel aspects:

A novel approach for the analysis of dioxin-like substances in environmental and food samples based on the use of GC-QqQ(MS/MS) is compared to reference techniques based on HRMS.
In-line preconcentration capillary electrophoresis-electrospray ionization-mass spectrometry for the analysis of haloacetic acids in tap water

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Keywords: haloacetic acids, in-line preconcentration, CE-MS, tap water

Haloacetic acids (HAAs) belong to an important class of disinfection byproducts that are being regulated. A strategy based on in-line preconcentration CE-ESI-MS/MS was developed for determination of haloacetic acids (HAAs) in tap water. The field amplified sample injection (FASI) technique was used for in-line preconcentration of five HAAs including monoacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), dibromoacetic acid (DBAA) and monobromoacetic acid (MBAA). In FASI-CE-MS/MS analysis, 50% MeOH containing 2.5% ammonium acetate (pH 3.5) was used as background electrolyte and sheath liquid. Low sheath flow interface was used to preserve separation condition and to stabilize electrospray ionization process. With 30 second hydrodynamic injection of a water plug followed by 20 second electrokinetic injection of the sample, FASI enrichment factors in a range of 1000-3000 were obtained. Detection limits of HAAs were in a range of 0.01-0.1 μgL-1. In tap water analysis, three HAAs including TCAA, DCAA and MBAA were detected at a concentration about 0.1-1ppb, 0.1-1ppb and 0-0.1ppb, respectively. The feasibility of FASI-CE-ESI-MS/MS in real time analysis is demonstrated by continuous monitoring HAAs in tap water.

Novel aspects:
A simple and sensitive FASI-CE-ESI-MS/MS method was developed for in-line monitoring haloacetic acids in tap water.
Effects of silver on Chlamydomonas reinhardtii; insights from proteome analysis and physiological endpoints

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Keywords: proteomics, silver, Chlamydomonas reinhardtii, ecotoxicology

Classic ecotoxicology has focused upon a bottom-up approach to understand stressor effects in which a few genes, proteins, or biochemical reactions are studied at a time. The invention of new technologies in the last decade has enabled the analysis of whole transcriptome (gene transcripts), proteome (proteins) and small cellular molecules (metabolite profiling) resulting in whole system approaches. By complementing the traditional approach with the system biology it is possible to define the genetic, protein, and biochemical reactions as integrated and interacting networks of an organism. We use such a systems biology approach to understand the biological responses of Chlamydomonas reinhardtii to ionic silver, which is toxic to a variety of aquatic organisms in the nanomolar range. Specifically, the proteome profiling by Multidimensional Protein Identification Technology (MudPIT) allows determination of differential expression of proteins. MudPIT analysis of C. reinhardtii exposed to silver allowed discrete identification of roughly 2500 proteins in each sample with false discovery rate set to 2%, representing major cellular processes. Enrichment analysis showed significant regulation of several biological pathways. Key among them were photosynthesis, ATP synthesis and tetrapyrrole synthesis with all being severely down-regulated. Differently, some pathways that were up-regulated were the lipid synthesis, oxidative stress response, proteolysis and cell wall synthesis. Our results provide the first insights into the mechanisms of toxicity of ionic silver. Silver is taken up into the cells via active metal transporters and inhibits key proteins involved in photosynthesis and ATP synthesis. Silver also induces oxidative stress as deduced from the induction of oxidative stress response proteins such as GPXH. The up-regulation of lipid synthesis also indicates an autophagy response. Importantly, we could link the changes at the proteome to the physiological state of the algae on exposure to silver.

Novel aspects:

systems biology view of silver-induced stress response in green algae. Linking of molecular responses to physiological effects
Investigating the origin of the enigmatic fairy circles of Namibian by using a simple silicone rubber sampling device and GCxGC-TOFMS

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Keywords: Comprehensive GCxGC-TOFMS; Polydimethylsiloxane (PDMS) sorptive extraction; Fairy Circle; Natural gas; Geochemical

A striking feature of the landscape of the Pro-Namib in southern Africa is the so called fairy circles - large barren patches visible in the western grasslands in which no vegetation grow. These mysterious pock marks appear in southern Angola, continue through Namibia, and extend into South Africa. While highly visible in grasslands fairy circles are not exclusive to vegetated areas of the region. Various theories proposed as to the cause of the fairy circles are insect activity, localised radioactivity and toxins released by dead Euphorbia damarana plants, all of which lack conclusive evidence.

A fairy circle originates as a small circular zone of severely stressed, but wholly intact, plants. The stressed plants do not survive and eventually a large barren soil patch develops. After rain seedlings may appear in the patch, show stunted growth and do not survive. An in situ study revealed the presence of an active factor permeating up from below the soil surface within fairy circles. This seep factor was absent outside of circles. Furthermore, chemical alteration of fairy circle soils were indicated by potting trials showing that even in the laboratory plants exhibited stunted growth and mortality when planted in soil collected from within fairy circles. In contrast, plants flourished in soil collected from outside of circles. We developed a novel, cheap and simple soil sampling procedure where a length of silicone rubber (polydimethylsiloxane (PDMS)) tubing was fashioned into a loop. PDMS loops were placed in soil samples collected from inside and from outside of fairy circles. Chemicals absorbed from the soil were thermally desorbed from the PDMS loops into a comprehensive two dimensional gas chromatograph coupled to a time of flight mass spectrometer (GCxGC-TOFMS) for compound separation and identification. Chemical profiles thus obtained differed depending on whether the soils were obtained from within or from outside of fairy circles.

Low volatility hydrocarbons, and the geochemical biomarkers pristane and phytane, were detected in the soils of newly developed fairy circles. The finding supports our hypothesis of natural gas from below causing the stunted vegetation growth, in other words a geochemical origin of the Namibian fairy circles.

Novel aspects:
Novel, simple, cheap silicone rubber sampling device for soil; resolving unresolved complex mixtures by GCxGC-TOFMS; Geochemical natural gas origin of the fairy circles of Namibia, southern Africa
on-site analysis of gases emitted from soils using MULTUM-S II

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Keywords: on-site analysis, N\textsubscript{2}O

Recently, miniature mass spectrometers which can be used for on-site analysis have been designed and developed. These instruments are expected to have widespread applications. In our laboratory, a miniaturized multi-turn time-of-flight mass spectrometer "MULTUM-S II" was designed and constructed. Although the size of newly developed "MULTUM-S II " was 45 cm x 25 cm x 64 cm, it has capability of high mass resolution of more than 30,000.

We attempted to carry out real-time monitoring of the microbial production in the soil and consumption of gaseous compounds (N\textsubscript{2}O, CO\textsubscript{2}, CO and CH\textsubscript{4}) by using this MULTUM-S II. In this study, we attempted to monitor of N\textsubscript{2}O concentration in real-time. N\textsubscript{2}O is known as one of the important greenhouse gases, and its warming effect is 298 times higher than that of CO\textsubscript{2}. Atmospheric N\textsubscript{2}O concentration level is 314ppb and increases to several hundred ppm by bacterial activities. N\textsubscript{2}O is mainly produced by N fertilizer in agricultural soils. Therefore, on-site high performance N\textsubscript{2}O analyzer has been required to elucidate generating mechanism and estimate reduction procedure of generated N\textsubscript{2}O from agricultural soils. However, N\textsubscript{2}O is measured by using a gas chromatography (GC) with an electron capture detector (ECD) so that it is difficult to apply GC/ECD on the field due to the radioisotope (\textsuperscript{63}Ni) in the detector. On the other hand, we can measure N\textsubscript{2}O in the field by GC/MS. In this method, it takes about ten minutes to separate CO\textsubscript{2} and N\textsubscript{2}O in a separation column. If we could separate CO\textsubscript{2} and N\textsubscript{2}O by using a high-mass resolution mass spectrometer, the measurement time would be reduced significantly. However, required mass resolution which is to separate CO\textsubscript{2} and N\textsubscript{2}O doublet is larger than 8,000 due to the mass difference is 0.0113 u. Therefore, conventional miniaturized instrument can’t be separate the doublet. MULTUM-S II can provide high mass resolution in the compact size. Therefore, we attempted to separate CO\textsubscript{2} and N\textsubscript{2}O doublet. In this experiment, we used 5m PLOT column to separate N\textsubscript{2}O, CO\textsubscript{2} and main components of air (N\textsubscript{2} and O\textsubscript{2}). When large amounts of N\textsubscript{2} and O\textsubscript{2} are injected into the ion source at the same time, low amount N\textsubscript{2}O signal is suppressed. Using this procedure, we detected N\textsubscript{2}O in the atmospheric concentration level (314ppb). This result was significant increase compared to direct sample injection. Furthermore, the measurement time was within one minute. We also developed an automatic sampler to inject these gases into MULTUM-S II in one minute intervals for automatic real-time monitoring. As a result, real-time monitoring of atmospheric N\textsubscript{2}O concentration and its variation of emission from soils can be achieved by the combination of the automatic sampler and the MULTUM-S II.

Novel aspects:
We detected N\textsubscript{2}O in the atmospheric concentration level (314ppb) by using the MULTUM-S II within one minute.
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). R² 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/m³, 0.23 pg/m³ and 0.34 pg/m³, 0.36 pg/m³ 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/m³. Recoveries of ¹³C 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.
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 for 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 C18 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 urinary 1-OHNap, 2-OHNap, 1-OHPhe, 3-OHPhe, 4-OHPhe and 1-OHPyr were 1.6, 2.2, 0.2, 0.2, 0.1 and 0.2 μg/g creatinine in engineers and 0.5, 1.8, 0.2, 0.1, 0.1 and 0.1 μg/g creatinine in other crews, respectively. The method can be used to evaluate occupational exposure to PAHs.

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.
Thermal solid phase extraction for GC-MS analysis of complex samples

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Keywords: GC-MS, thermal desorption, solid phase extraction, sample preparation

Introduction
Pristine samples or sample extracts often contain matrix compounds and/or solvents, which are incompatible with gas chromatographic analysis. Thus, an often complicated and time-consuming sample preparation with potential loss of analytes is needed before analysis. Here we present thermal solid phase extraction (TSPE) as a simple and fast approach based on thermal desorption (TD) for analysis of samples in complex matrices. Samples are simply injected onto Tenax TA adsorbent tube and solvents (e.g. methanol, ethanol or water) are subsequently removed by purging with helium at ambient temperature. VOCs and SVOCs with boiling points up to ca. 400 °C are released during the following thermal desorption whereas larger and non-volatile compounds (e.g. sugars, proteins, fat, salts etc.) are retained by the adsorbent. Since TSPE may also be used for concentration of trace level samples by large volume injection onto the adsorbent, it can replace the following sample preparation steps:

1) Removal of unwanted matrix compounds that deteriorate the analysis
2) Solvent change to a suitable GC solvent
3) Up-concentration (e.g. by evaporation) of analytes before GC-MS analysis

Here TSPE has successfully been applied to the analysis of whisky and extracts of floor dust and tattoo inks.

Experimental
Single malt whiskies or methanol extracts of floor dust and tattoo inks were injected into stainless steel tubes containing Tenax TA adsorbent. The injections were followed by purging with He (60 ml/min for 3-5 min). injection volumes were 1-50 µl. A thermal desorber (Perkin-Elmer ATD 400) was connected to a GC-MS system (Perkin-Elmer Autosystem XL GC/TurboMass MS or Varian CP 3800 GC/1200 MS). The Tenax TA tubes were desorbed for 20 min at 250 °C, using a He flow of 50 mL/min, and a cold trap temperature of -30 °C. The cold trap was narrow bore and packed with Tenax TA for VOCs and empty for SVOCs. Flash heating of the cold trap to 300 °C transferred the analytes through the transfer line (225 °C) to the GC equipped with a 30 m VF-5ms column. The mass spectrometers were operated in electron ionization mode using full-scan (m/z 50-400).

Preliminary results
TSPE showed good chromatographic performance and minimization of co-eluting peaks. So far the method has showed to be quantitative within the range of VOC/SVOC from toluene to di-2-ethylhexyl phthalate (DEHP). The limit of detection of DEHP was estimated to 31 ng determined as three times the standard deviation of the mean of a low-concentration standard.

Whisky: For comparison several different whiskies brands (e.g. Ardbeg, Macallan and Glenlivet) were analyzed. The resulting chromatograms showed good chromatographic resolution and clear differences, thus facilitating (easy) distinction of the whiskies from one another. Phenols, cresols, acids and esters were identified as the main eluting compounds.

Floor dust: Comparison of floor dust samples from different locations in Denmark showed that the main constituents were phthalates (mainly DEHP), fatty acids and hydrocarbons. The main problem for direct on-column injection of floor dust extracts are the high content of fat. This problem was eliminated by TSPE.

Tattoo inks: A series of black tattoo inks (high content of carbon black pigment) were screened and compared for their content of organic compounds. They contained a large number of compounds including butanediol, phenol, trichlorobenzen, Texanol, fatty acid methyl esters, phthalates and PAHs.

The range of useful solvents is limited by the properties of Tenax TA to solvents like methanol, pentane and water. However, this may be extended to other solvents by use of other adsorbents.

TSPE has a large potential as a fast analytical method with minimal sample preparation for volatile and semi-volatile...
organic compounds in a wide range of sample matrices.

**Novel aspects:**
Thermal solid phase extraction (TSPE) is a fast and simple sample preparation for elimination of unwanted matrix compounds in GC-MS analysis of VOC and SVOC in complex matrices.
Organic coatings of engineered nanomaterials characterized by mass spectrometry

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Introduction

Engineered nanomaterials (ENM) are often chemically surface modified in order to tailor their physical-chemical properties for specific applications. For example, lipophilic surfaces are needed for incorporation of ENM in polymers whereas hydrophilic surfaces are used in water-based paints. These surface modifications may have influence on the toxicological and environmental properties of the ENM, but they are often trade secrets. Therefore, analytical procedures are needed to reliably enable detection and quantification of unknown ENM surface modifications. Only a limited number of publications describe quantitative methods for this type of application and despite of the obvious advantage of using mass spectrometry (MS) only a few studies describe the use of MS. Here we present an approach, based on several MS techniques, to characterize high temperature extractable and predominantly non-covalent bounded organic coatings of ENM.

Experimental

Thermogravimetric analysis (TGA) was used to identify ENM with organic coating. The following ENM with more than 1 wt% apparent coating were investigated: Organoclays, graphite, synthetic amorphous silica, titanium dioxide, silver, calcium carbonate, iron oxide, and nickel-zink-iron oxide. The ENM were extracted with pressurized liquid extraction (PLE) using methanol at 200 °C and 140 bar. The extract was centrifuged and the supernatant used for the succeeding analysis.

MS combined with on-column gas chromatography (GC-MS) or thermal desorption was used to analyze the extracts for volatile organic compounds. A 30 m 5% phenylmethylsilicone column was used for GC and the MS was run in electron ionization (EI) mode and the mass range was m/z 50 to 500. The identification of organic compounds was based on searches in the NIST 2011 MS library and authentic standards.

Quadrupole time-of-flight MS combined with liquid chromatography (LC-MS) or direct infusion was used for analysis of non-volatile organic compounds in the extracts using both electrospray ionization and atmospheric pressure chemical ionization. The MS was operated in positive mode and the mass range was usually m/z 50 to 3000. Collision induced dissociation was used for structure determination.

Nanostructured surface-assisted laser desorption ionization time-of-flight MS (NALDI-TOF) was used for characterization of polymeric compounds. 1 µl of either a suspension of the ENM in methanol or the PLE extract was deposited on the NALDI target. Sodium trifluoro acetate was used as cationizing agent. Spectra were acquired in the reflector-positive mode and mass range was m/z 50-5000. The identification of the organic compounds from the NALDI-TOF results was based on pattern recognition, literature, and GC-MS data.

Results

A wide range of organic compounds was extractable from the ENM and their identity was confirmed by authentic standards in several cases. For example organoclays were surface functionalized with quaternary ammonium compounds containing benzyl and alkyl side chains to make them miscible with polymers. Silver was surface modified by 2-pyrrolidone or coated with polyethoxylated nonionic surfactants. Graphite, calcium carbonates, iron oxides were coated with a mixture of fatty acids and fatty acid methyl esters - probably added to prevent agglomeration. Other compounds were silanes and siloxanes from synthetic amorphous silica and nickel-zink-iron oxide. Most of the extracted compounds are presumably intentionally added for different purposes. Some of the surface modifications have already been shown to influence the toxic properties of the ENM.

Novel aspects:

This is the first general approach using mass spectrometry for quantitative characterization of organic surface modifications of engineered nanomaterials.
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: monomethyl- (MMP, 0.203), monoethyl (MEP, 0.162), monon-butyl (MnP, 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-ethylhexyl)- (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.
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 “1st column”) is introduced (heart-cut) to a column of another type (called the “2nd column”), so that insufficiently separated components can be separated. A device called a ”switching device” is used for heart-cut introduction of peaks eluted from the 1st column to the 2nd column. As a switching device, the recently developed Multi-Deans switching unit can be used in combination with a GC-FID as the first analytical dimension and a GCMS as the second analytical dimension. The analytes pass the first column and are detected in the FID (“stand-by mode”) or are transferred to the second column and analyzed with mass spectrometer or GC detector such as FID (“cut mode”). By using this system, complicated matrix analysis such as hydrocarbon mixture was done to demonstrate MDGC/GCMS system performance. This MDGC/GCMS system can analyze the olefine and aromatic hydrocarbon in hydrocarbon mixture. It has superiority on the determination of the complicated matrix sample in order to obtain more reliable analytical results.

Novel aspects:
MDGC/GCMS system can show superiority on the analysis of complicated matrix sample such as hydrocarbon mixture, etc.
Powerful GC-ToF-MS Techniques for Quantification of Legacy Pollutants and Screening and Identification of Emerging Pollutants

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Keywords: GC × GC, multi-reflectron ToF, PCBs, dioxins, emerging pollutants

Environmental samples often present a challenging task to the analytical chemists. The target compounds are generally present at trace levels in a complex cocktail of natural and anthropogenic compounds. In order to accurately quantify such trace compounds it is essential to reduce the background, but also to efficiently separate the target compounds from each other and residual matrix. Although some advances have been made in the streamlining of sample preparation, e.g. using simultaneous extraction and clean-up, the focus on this talk will be on powerful GC-MS methods for quantitative and qualitative analysis of legacy and emerging pollutants.

Over the last decades, comprehensive two-dimensional GC (GC × GC) has evolved into a robust and exceptionally powerful technique for group-type as well as within-group (isomer) separations. Group-type separations of petroleum hydrocarbons were one of the first and most important areas of application. In the case of isomer separations, one of the more demanding tasks is the complete separation of the 209 PCBs or 210 polychlorinated dioxins and furans (PCDD/Fs). Although the quest still continues, it has been possible to greatly enhance the separation. It is now possible to separate all dioxin-like PCBs from each other and from other PCBs, and separate all 2,3,7,8-PCDD/Fs from each other and from most other PCDD/Fs. It is also possible to perform enantioselective analysis of PCB atropisomers (asymmetric tri- and tetra-ortho PCBs). However, careful selection and optimization of the column sets are essential. For PCB and PCDD/F analysis the coupling of a long efficient non-polar first and a shape-selective liquid crystal second dimension column proved to be most successful; while for the atropisomers, the first column had to be changed to a permethylated cyclodextrin column. In these separations, the target compounds were more and less retained, respectively, by the liquid crystal phase than the potential interferences.

The high peak capacity of GC × GC ToF MS can also be utilized to perform comprehensive characterization of complex samples, e.g. extracts of soil from contaminated sites or water from sewage treatment plants (STPs). However, the number of components detected and tentatively identified in such non-target analyses are overwhelming and logical and efficient prioritization tools are required to make such studies feasible. Recently, two different approaches were tested: a risk-based prioritization and a property-based classification. In the first, quantitative structure-activity relationships were applied to all tentatively identified compounds in contaminated soil extracts and the measured concentrations were compared to the estimated no-effect levels to obtain a risk-ratio. All compounds with high risk ratios were prioritized for confirmatory analysis and, if confirmed, further analyzed in biota potentially affected by soil contaminants. The property based classification was also based on ration calculations, i.e. the concentration ratio of STP effluent and influent water. Compounds with ratios much lower than 1 are removed to a great extend, those with a ratio close to 1 are poorly removed, and those with a ratio above may actually be formed in the treatment process. In this way, compounds that are classified as poorly removed or metabolites may be identified, which will aid in the improvement of existing and design of new STP processes.

Although ultimate confirmation requires authentic standards novel GC-MS techniques such as ultra-high resolution ToF-MS may be used to provide valuable information. Such techniques deliver full-scan spectra at a resolution up to 50,000 and a mass precision of 1 ppm, which is sufficient to unambiguously determine elemental compositions of ions up to ca 150 amu (may be extended to ca. 500 amu using isotope abundance analysis). This is generally sufficient to confirm or reject tentative structures and greatly enhances the possibility to perform a correct manual interpretation.

Novel aspects:
Two-dimensional gas chromatography and ultra high-resolution mass spectrometry provide exceptional peak capacity, mass resolution and mass precision and open new possibilities in environmental analyses.
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 1H-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 an 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.
Analysis of perfluorinated compounds in sediment samples from wastewater canal of Pancevo industrial area, Serbia

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Keywords: PFOA, PFOS, Industrial wastewater canal, Sediment, Danube River

Perfluorinated compounds (PFCs) are chemicals that do not occur naturally, but have been widely used in chemical production for some time. They are globally distributed, environmentally persistent, bioaccumulative, and potentially harmful. Perfluorooctansulfonate (PFOS) and perfluorooctanoate (PFOA) are the two PFCs most commonly used and found in the environment. Together with perfluorohexane sulfonate (PFHxS) these compounds are widely employed in different industrial processes such as in protective coatings.

The wastewater canal (WWC) Vojlovica was built in 1962 to collect the wastewater discharges from the industrial complex of the city of Pancevo in Serbia. Industrial complex consist of a petrochemical factory (HIP Petrohemija), an oil refinery (NIS Rafinerija, Pancevo) and chemical fertilizers factory (HIP Azotara). The canal is artificial with no natural flows, about 2 km long, around 70 m wide and directly connected to the Danube River. The water depth is around 12 m. The environment surrounding the canal has been strongly affected for a long time by the presence of the industrial complex. Additionally heavy destruction during NATO bombing events in 1999 resulted in contamination of air, soil, groundwater and the WWC itself.

In total, 4 sediment samples from WWC were collected. Surface sediments layer of 15 cm were taken by a Van Veen Grab sampler, transported in glass jars and stored in the laboratory at 4°C. For comparative purposes, the same type of sample were also taken from the navigation canal flowing parallel to WWC but not receiving any direct discharge of industrial wastewaters.

Sampling sites are listed below:
No1 - navigation canal;
No2 - at the confluence of WWC with the Danube River, downstream from the industrial area and effluents;
No3 - downstream from the fertilizer factory outlet (first effluent);
No4 - downstream from the petrochemical plant (second effluent);
No5 - downstream from the oil refinery outlet (third effluent).

Sediment sample was extracted with methanol. MPFAC-MXA as mass-labeled surrogates was spiked into the sample. The sample was extracted with SPE. The elution was concentrated and labeled ¹³C₈PFOA was added as syringe spike. The each final solution was analyzed by liquid chromatography (LC)-tandem mass spectrometer (MS/MS) using Xevo TQ (Waters) coupled with ACQUITY UPLC (Waters).

Concentrations of PFCs were determined as follows:
No1: 68, 230 and 230 ng/kg-dry of PFOA, PFHxS and PFOS, respectively.
No2: 80 and 2100 ng/kg-dry of PFOA and PFOS, respectively.
No3: 170 and 5300 ng/kg-dry of PFHxS and PFOS, respectively.
No4: 130, 170, and 5700 ng/kg-dry of PFOA, PFHxA, and PFOS, respectively.
No5: 76, 66 and 420 ng/kg-dry of PFOA, PFHxA, and PFOS, respectively.

Concentrations of PFOS in the samples No3 and No4 are 3-3.2 times higher compared with sea sediment in Tokyo bay.¹ PFOA and PFOS concentrations from WWC were from two to twenty fold higher comparing to sediment samples taken from Roter Main river (Germany) which receives treated waste waters of industrial, commercial and domestic origin from municipal wastewater treatment plant.² Comparing to upstream Danube River bank sediment samples ³ PFOS from the WWC samples were from two to six fold higher.

This is the first study and report of presence of PFCs compounds in the samples from Serbia. Most of the PFCs are released from fertilizer factory and petrochemical plant outlets, while oil refinery outlet mostly contribute to petroleum pollution. The exact origin of PFCs cannot be established from one study but one of the reasons for presence of these compounds might be their usage as components in pipes, fittings and wiring insulations.

1) Zushi Y. et al Environmental pollution 158, 756-763 (2010)
2) Becker, A.M et al Environmental Pollution 156, 818-820(2008)
Novel aspects:
This is the first report of presence of PFCs compounds in the sediments from Serbia. Compared to other reports, high levels of PFOA and PFOS were found.
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 pyrethroid insecticide, was studied by using a chemical oxidation process and the degradation products were monitored by using SBSE probe desorption GC-IT-MS. Degradation was performed by using 50% hydromethanolic solution containing 45 mg L⁻¹ esfenvalerate, pH 11.25, 25 mg L⁻¹ of hydrogen peroxide, within a 4 hour reaction period. After degradation process, the products were extracted by SBSE by using stir bars (10mm × 0.5mm, 24 µL PDMS coating, Twister, Gerstel) at room temperature (25°C), for 120 min, with 15% methanol, 12% NaCl and stirring at 1000 rpm. After extraction, the stir bar was placed into the probe of a GC CP 3800 (Varian), coupled to an ion trap MS Saturn 2000 (Varian). Thermal desorption of the analytes were done at 250°C, and the probe heating was held for 18 min. GC analysis were performed on a DB-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°C (held for 0.5 min) to 300°C/min (held for 6 min), at 20°C/min. Helium was used as carrier gas at flow rate of 1.2 mL min⁻¹. The MS analyses were done in the scan mode (m/z 40 to 450) using electron impact ionization (70 eV). The temperature of transfer line, ion trap and manifold were set at 300°C, 220°C and 40°C, respectively. By using the SBSE probe desorption-GC-IT-MS method, it was possible to fully identify two products of chemical oxidation of esfenvalerate, 3-phenobenzoic acid and 3-phenoxbenzaldehyde. These compounds were previously described as being metabolites of esfenvalerate, with small estrogenic (endocrine-disrupting) activity and possibly with small environmental impact (McCarthy et al., J. Environ. Monit. 2006, 8, 197). The structural elucidation of the other oxidation products of esfenvalerate is still in progress.

Acknowledgements: FAPESP, CNPq

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.
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 character 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.
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-milliliter portions of samples were taken from each stirred sample once a week for a month. The portions passed through solid phase extraction cartridges, OASIS W AX (Waters), to extract PFCs. PFCs were eluted from the cartridges with 0.1% NH₄OH methanol. The eluates were analyzed by liquid chromatograph/mass spectrometer, Ultimate 3000/Exactive (ThermoFisher). Ultimate 3000 and Exactive were used for acquisition of mass spectra with a high resolving power of 100,000. In addition, Acquity and Xevo TQ (Waters) were used for quantitation of PFCAs and PFSAs and acquisition of product ion spectra. Two-dimensional LC was carried out with two different kinds of columns. A TSK-Gel ODS-100S from TOSOH and an Epic-FO column from ES-industries were used for chromatography.

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

The chemical structures were determined from the interpretation of the data of samples before biodegradation. A dominant ion with an m/z value of 1574.2809 was found in one product examined. Because three fragment ions that had equal spaces of 357.007 were found in the observation with collision gas, the compound was the molecule that had three \( \text{C}_4\text{F}_9\text{SO}_2\text{NCH}_3\text{C}_2\text{H}_4\text{O} \) groups. If triisocyanate such as Tolonate reacts with an alcohol, \( \text{C}_4\text{F}_9\text{SO}_2\text{NCH}_3\text{C}_2\text{H}_4\text{OH} \), the chemical formula of the molecule yielded will be \( \text{C}_{45}\text{H}_{60}\text{N}_9\text{O}_{15}\text{S}_3\text{F}_{27} \). 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.
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 in lubricants and hydraulic fluids etc. are the useful chemical compounds and are used in the various areas. It is well known that aquatic environment has contaminated by volatilization, leaching or abrasion from the broad application range of these compounds. In this study, the concentrations of eight species of organophosphate esters (OPE) and OP pesticides (diazinon, fenitrothion, chlorpyriphos and iprobenphos) are measured in water, sediment and green mussels from Maizuru Bay and the distribution of these compounds in water environment is discussed.

Water sediment and mussel samples in Maizuru Bay were taken from July 7th to 15th, 2009. Subsurface water samples were taken in 7 sites and the surface sediment samples (0.5 cm) were taken using a Ekman-Birge type bottom sampler in 13 sites. The mussel (\textit{Mytilus galloprovincialis}) samples were taken at 9 sites. The shell lengths of the green mussels were in the range of 380 - 750 mm. Three mussel samples in each site were homogenated before analysis. Water samples were stored in a fridge at \(3\)\(^{\circ}\)C and their samples were analyzed within a few day. Sediment and mussel samples were stored in a freezer at \(-20\)\(^{\circ}\)C for until chemical analysis.

Water samples were extracted with dichloromethane by shaking for 10 min. The aqueous layer was dried with anhydrous \(\text{Na}_2\text{SO}_4\) and was concentrated up to 0.5 ml after the addition of Atradine-d\textsubscript{25} as an internal standard. The analytes were measured by GC-MS. OPs in sediment and mussel samples were extracted with acetone. After centrifugation, the supernatants were cleaned by addition of distilled water, celite and zincacetate. After removal of suspended matters by filtration, the analytes in liquid layer were extracted dichloromethane. After the addition of Atradine-d\textsubscript{25}, the organic layer was concentrated up to 0.5 mL. The analytes were measured by GC/MS.

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

The concentrations of OP compounds in sediment from Maizuru Bay were in the range of <0.5 - 56 \(\mu\)g kg\(^{-1}\) dry weight (dw). The patterns of OPEs in sediment are classified two groups. The patterns of OPEs concentrations in a group are characterized that OPEs concentrations are high the order of TBXP > TDCPP >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 \(\mu\)g kg\(^{-1}\) dry weight (dw). The patterns of OPEs in sediment are classified two groups. The patterns of OPEs concentrations in a group are characterized that OPEs concentrations are high the order of TBXP > TDCPP >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 partition rate between water and sediment (K\textsubscript{ws}) of diazinon and fenitrothion were in the range of 200 and 1300, and in the range of 200 and 300, respectively and the partition rate between water and biological samples (K\textsubscript{wb}) 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.
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 determination of three endocrine disrupters (estradiol, ethinyl estradiol, and bisphenol A) and five pharmaceuticals (sulfamethoxazole, trimethoprim, diclofenac, bezafibrate and miconazole) in raw and treated sewage samples was developed and validated. The method consisted of the application of tandem SPE procedure that uses both a strong ion exchange sorbent (SAX) and a modified divinylbenzene-pyrrolidone sorbent to reduce the levels of linear alkylbenzenesulphonate (LAS) and to concentrate the analytes of interest from sewage samples, prior to analysis by liquid chromatography combined with high-resolution mass spectrometry (LC-HRMS). The influence of matrix composition on the ionization efficiency, the SPE recoveries, and the sensitivity of the method was determined. The SAX sorbent was capable of significantly removing LAS content in sewage samples extracts. It was also capable of retaining the analytes that were eluted with ethyl acetate with recoveries that varied from 17 to 35% (CV <9%). This approach was very efficient at minimizing the matrix effects. The sum of the recoveries from both sorbents varied from 30 to 70% (CV <9%).

The mass spectrometry detection was performed using a LC-ESI-IT-TOF/MS instrument working at high-resolution (10,000 FWHM) and high mass accuracy (< 5 ppm). The instrumental limits of quantitation varied from 0.4 µg L⁻¹ to 3.3 µg L⁻¹. The method limits of quantitation ranged from 3.3 ng L⁻¹ to 41 ng L⁻¹. The method was successfully applied to the determination of analytes in raw sewage samples at the Arrudas Sewage Treatment Plant, Belo Horizonte, Brazil and also to evaluate the efficiency of different experimental sewage treatment systems.


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.
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 Agilent 7500c 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.
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. H₂O and CO₂). 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
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: LC-MS-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 analyse 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 (R² > 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.
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

\section*{Introduction}

In recent years, many kinds of chemicals are polluting environmental water. When people take a medicine, use insect repellent, or apply cosmetics, these chemicals flows into the water in the environment, finally. These compounds are called PPCP (Pharmaceuticals and Personal Care Products) as a whole. Furthermore, it is known that the pesticides originally used for agricultural products are polluting environmental water. These compounds are also contained in PPCP in a broad sense.

The diversity and the numerousness of these compounds make method development challenging. But in order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is able to analyze polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range, such as pesticides, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products etc. In addition, state-of-the-art LC-MS/MS instruments operated in selective Multiple Reaction Monitoring (MRM) mode, offer unmatched selectivity and sensitivity to quantify PPCP reproducibly at trace levels without time consuming and extensive sample preparation.

AB SCIEX QTRAP\textsuperscript{®} 4500 LC/MS/MS System can measure many compounds simultaneously because of its fast MRM scanning, and its can measure compounds high-sensitive because its has Q-jet \textsuperscript{®} 2 ion guide.

More recently, it is discussed that detection only by MRM cause false positive and false negative. To avoid false detection, one useful process is library search for a fragment ion spectrum. This process judges positive or negative by comparing the spectrum of standard with the spectrum of the compound detected in the sample. At 1 ch MRM, while judging from one fragment ion, by the library searching a spectrum, a judgment is performed from all fragment ions which the compound has. Therefore, library search can do more precise judging.

AB SCIEX QTRAP\textsuperscript{®} 4500 LC/MS/MS System can acquire fragment ion spectrum fast and sensitive because its has Linear Ion Trap technology with Linear Accelerator\textsuperscript{TM} Trap. And AB SCIEX has fragment ion spectrum library that contains large number of compounds.

LC-MS/MS is suitable for measurement of PPCP, and judgment by library search is desirable. Therefore, AB SCIEX QTRAP\textsuperscript{®} 4500 LC/MS/MS System suitable for PPCP screening.

\section*{Methods and results}

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

From Japanese environmental water, some compounds were detected by MRM. The detected compounds did not related sampling point. According to results of the library search, some compounds were truly detected, but some compounds were false positive. As a conclusion, the PPCP exist Japanese wide area environmental water. So it is necessary to measure much more samples to know a trend, such as regionality.

\textbf{Novel aspects:}

Fast Acquisition time, High Sensitivity, Direct measurement, Over 100 compounds
**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, perfluorinated compounds (PFCs) have appeared as a new class of global contaminants. Researchers have reported PFCs contamination in surface water, tap water and bottled water around the world. They are particularly difficult to deal with once released into the environment, because they do not break down easily, they can travel long distances carried by air or in water and they can accumulate in human and animal tissue. To understand the sources and fate of these compounds, it is essential to optimize the analytical methods for a wide range of perfluorinated compounds in water sample. Solid phase extraction (SPE) coupled with high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) is generally used to analyze PFCs in environmental matrices. Researchers have reported the difficulties in analysis of short chain (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 optimization of SPE process is needed. The objective of this study was to evaluate the optimum SPE condition for two ion exchange (OasisWAX and PresepPFC-II) cartridges for analyzing 12 PFCs in water sample. These cartridges were also compared with PresepC-Agri (C₁₈) 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 (C₁₈) 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 extraction was optimized for analysis of twelve PFCs in water sample, especially tap water and river water (with low matrix).
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 polyurethane 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 m³). After collection was completed, clean-up spike (OH-Di-Hp-CB-13C12 3.0 ng) 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 to 100 μL and added syringe spike (MBP-70-13C12 0.5 ng). The method detection limits (MDL) and the method quantification limit (MQL) were 0.067 - 0.13 and 0.17 - 0.33 pg/m³, respectively. The average of recoveries (n = 3) from 1000 m³ of air sample added with 3.0 ng OH-PCBs were almost 60-110%, and the relative standard division was 1.2 - 24%. Lowly chlorinated OH-PCBs tend to be collected in PUF and highly ones tend to be collected in QFF. In the air samples, OH-PCBs were detected 1 pg/m³ 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.
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.
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 polychlorinated dibenzo-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.
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.
Analysis of biomarkers of the pesticide imazalil using LC/MS/MS.

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

Introduction - Imazalil is a widely used post-harvest fungicide applied on a variety of crops, especially on fruits. Imazalil is also used in veterinary medicine as an antimycotic drug. Imazalil is classified as "Likely to be carcinogenic in humans," according to EPA. Exposure to imidazole derivatives is also suspected to produce craniofacial malformations in vertebrate development. Studies on rats have shown that imazalil is metabolized into 25 different metabolites with 1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)-1-ethanol (DCPI) as the major metabolite. There are very few studies in humans, however a case study of a patient treated with imazalil indicated a half-time of 2 h in serum. Various methods have been published for determination of imazalil residues in agricultural and food products. However, no methods have been described for determination of imazalil or its metabolites in human biological samples. Human occupational exposure can be substantial especially in developing countries where the use is high. It is especially common that women in fertile age are exposed during post-harvest treatment. Thus, methods for measurement of biomarkers of exposure is valuable. The aim of this study was to develop a simple liquid chromatography-tandem mass spectrometry (LC/MS/MS) method for the analysis of imazalil and DCPI in human urine.

Method - Several SPE columns were evaluated and optimized for sample pretreatment. The eluates were analyzed using electrospray ionization and selected reaction monitoring (SRM) in positive ion mode after separation on a C18 LC column. The mobile phase used was water and methanol with 0.1% formic acid. D5-labeled imazalil was used as an internal standard.

Results - Two SPE columns could be used for sample pretreatment, C2 and Oasis HLB. The sample was retained at neutral pH and eluted using 1% formic acid in methanol. For quantification of imazalil the transition at m/z 297.1-159.0 and for DCPI the transition m/z 257.0-69.2 was used. The method have an LOD of 1 ng/ml for both imazalil and DCPI. Data on levels found in a population will be presented.

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.
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 (CH4) is the second most important long lived greenhouse gas. The total global CH4 source strength is well described, whereas substantial uncertainties exist about the strength of individual source components. When it was discovered that terrestrial plants emit CH4 under aerobic conditions it presented quite a conundrum as CH4 is a highly reduced molecule. The ubiquitous plant structural component pectin is a highly activated methyl donor, and in purified form it emits CH4 in response to UV-radiation. Therefore, pectin is regarded as one of the most likely precursors in plants to aerobically emitted CH4. In vivo, however, pectin is situated under the cuticle 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 CH4 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 (C1-C3) 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 CH4 emission rate of 153 – 14 nmol CH4 g-1 wax h-1 in air when exposed to UV-radiation. The GCMS analysis of the leaf wax revealed (C29H60) and 15-nonacosanone (C29H58O) 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 C14H30/C13H26 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 and photolytical studies has resulted in detailed understanding of the underlying mechanisms for aerobic formation of methane from plants.
**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 morphone by mixture of chloroform and isopropanol (9:1; v/v).

**HPLC/MS analysis**

Extracts were analyzed by HPLC-ESI-MS technique: at Waters Alliance system, the separation column Waters Spherisorb 5 μm, ODS2, 4.6 × 100 mm was used; mobile phase: ammonium acetate : acetonitrile (80:20; v/v), mobile phase flow rate 0.3 mL/min, autosampler temperature 20°C; injection volume 50 μL; Mass detection range: 100-400 m/z, centroid mode, interscan delay 0.1 s, scantime0.5 s, splitless, four voltage values: 70, 60, 50 i 38 V, ES+, source temperature 150°C, desolvation temperature 430°C, gas flow for desolvation 362 L/h and at cone 135 L/h, capillary voltage 3 kV - mass spectrometer Waters Micromass ZQ™ (Waters Corporation, Milford, MA, USA).

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

**Results and Discussion**

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

Applying the proposed method on real samples of patients (n=10) where positive reaction was obtained on test strips we determined concentrations of heroin metabolites in selected samples in following ranges: Concentration of codeine in the saliva of the heroin abusers ranged from 0.05 to 5.33, for morphine between 0.05 and 5.33 and for 6-mam between 0.01 and 0.68 mg/L. Concentration of codeine in the urine samples of the same patients ranged from 0.22 to 5.74, for morphine between 0.15 and 6.32 and for 6-mam between 0.05 and 1.78 mg/L.

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

**Novel aspects:**

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

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

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

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

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

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


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.
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 cruises 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 BGB 172 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 viewpoint 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 analysis, it is regarded that enantioenrichment indicates that it was released some time ago and has since been subject 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.
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 at least two biotransformation products corresponding to the deprotonated molecular ions \([M - H]^- = 251\) and \([M - H]^- = 267\) were present. Fragmentation analyses revealed that these compounds appeared to be ring-opened structures derived from initial angular dioxygenation of the dibenzo-p-dioxin molecule by strain KK22 and they were tentatively identified as 6-(2-hydroxyphenoxy)-6-oxo-hex-4-enoic acid and a singly-hydroxylated derivative respectively. If confirmed, these chemicals shall represent previously unreported metabolites from dibenzo-p-dioxin by a bacterium. Further investigation is continuing to determine the nature of dibenzo-p-dioxin transformation by strain KK22 through the utilization of LC/ESI(-)-MS/MS.

**Novel aspects:**

LC/ESI(-)-MS/MS was utilized to determine biotransformation products of dibenzo-p-dioxin by a bacterium.
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 α-chloroacetophenone as well as arsenic oil containing Clark I, triphenylarsine, phenylidichloroarsine and trichloroarsine, and related degradation products. After an appropriate sample preparation procedure, the samples were analysed using GC-EI/MS with selected ion monitoring (SIM) as such and after derivatisation with propanethiol, and LC-APCI/MS/MS with selected reaction monitoring (SRM) as such and after oxidation using hydrogen peroxide.

Analysed sediment samples showed considerable spreading of arsenic-containing chemicals within the dumpsite as well as between the dumpsite and the Bornholm Island. The highest found sediment concentrations have been over 16,000 ng/g of degradation products of Clark I and 39,000 ng/g of triphenylarsine within the primary dumpsite. [1]

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

References

Novel aspects:
Novel information of CWA contamination of the Baltic sea.
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) seem 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
Identification and quantification of concentration-dependent biomarkers in MCF-7/BOS cells exposed to 17beta-estradiol by 2-D DIGE and label-free proteomics

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Keywords: Biomarkers, estrogens, food-chain security, proteomics, MCF-7/BOS

The rapid screening of xenoestrogens prior to heavy analytical techniques is an important step in environmental pollution monitoring and food chain safety. We report the identification of biomarkers resulting from the exposure of MCF-7/BOS cells to 17beta-estradiol (E₂). The biomarkers were identified from the cytosolic fractions of cells treated for 24 h with mitogenic concentrations of 1, 30 and 500 pM of 17beta-estradiol. The biomarkers were identified using 2 independent and complementary techniques, 2-D DIGE / MALDI-TOF peptide mass fingerprint, and 2-D UPLC-ESI MS/MS. To enable the further addition of new chemicals in the study, the 2D-DIGE experiment were performed using, instead of the classical internal standard, a reference gel. A robust PCA analysis allowed to filter the biological variability and recover a dose dependence variation. Five biomarkers were up-regulated proteins, HSP 74, EF2, FKBP4, EF1 and GDIB and one was a down-regulated protein, K2C8. Three of these proteins, EF2, FKBP4 and K2C8 are implicated in a network centred on the estrogen receptors ESR1 and ESR2 as well as on AKT1. After the discovery phase, three biomarkers were selected as a signature for the response to the presence of estrogens. They were monitored using SRM after incubation of MCF-7/BOS in the presence of E₂ for confirmation or selected xenoestrogens. Daidzein, coumestrol and enterolactone induced an up-regulation of EF2 and FKBP4 proteins, while tamoxifen and resveratrol induced a down-regulation. The exposure of all phytoestrogens induced the down-regulation of K2C8. These markers form a preliminary molecular signature that can be used when testing the estrogenic activity of new chemicals, xenobiotics, either pure or in mixtures extracted from food chain or environmental samples.

Novel aspects:
2D-DIGE and label free proteomics coupled with PCA analysis allowed the discovery of estrogen activity biomarkers that can be used for rapid screening of estrogenic activity of xenobiotics.
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 anthocyanins profile was analyzed by mass spectrometry in this type of local Brasilian grape. This work successfully demonstrates the capability of MALDI-MS to analyze anthocyanins 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-hydroxycinnamic acid 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 anthocyanins profile of Bordô skin grape was evaluated and a new selective method for their mass spectrometric analysis is proposed.
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
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 in food revolve around using triple quadrupole platforms and long run times. The method described here utilizes UHPLC- with a second generation Exactive™ mass spectrometer (MS) using high resolution accurate mass. The second generation Exactive MS is capable of resolving powers settings of up to 140,000 (FWHM) at m/z 200, providing the ability to resolve matrix from analyte in full MS mode even in most complex matrices. This work describes a method to do screening and quantitation of a 100 pesticides mixture together with high level confirmation.

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

Preliminary Data:
Calibration lines were generated for the compounds analyzed with $r^2$ better than 0.9940, the limits of detection (LOD) varied from 50pg/mL to 500pg/ml based on the individual compounds. For confirmation of each compound the exact mass of the compound, its isotopes and as well as the AIF produced were collected and compared to limit the amount of "false positives" in the results. One of the main challenges using a high resolution accurate mass system is data mining, but with novel ExactFinder™ software the data processing becomes straightforward. Using the novel second generation Exactive the workflow to screen 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.
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 N₂-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 rubyretic 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-glycosides 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.
Dramatically Improved Hydrocarbons Analysis with the 5975-SMB GC-MS with Cold EI

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Keywords: GC-MS, Cold-EI, Wax, Hydrocarbons, Fuel-Oil

Hydrocarbon analysis, including fuels, oils and waxes are of vital importance to broad range of applications. However their analysis by GC-MS is confronted with limitations in the elution of large and low volatility compounds and in the absence of trustworthy molecular ions and representative high mass fragments which consequently often precludes proper identification. In addition, such analysis is further exacerbated by the lack of proper LC and/or LC-MS alternatives.

GC-MS with supersonic molecular beams (SMB) and its Cold Electron Ionization (Cold EI) allow to overcome the challenges of hydrocarbon analysis occurring in standard GC-MS technique. Cold EI with it fly-through ion source eliminates the well-known conflict between ion source related peak tailing and molecular ion abundance which originates from the conventional ion source layout as will be explained and discussed. In addition, the SMB based approach significantly increases the range of compounds amenable for GC-MS analysis via lowering the compounds elution temperatures as will be demonstrated.

We used an Aviv Analytical model 5975-SMB GC-MS with Supersonic Molecular Beams (SMB) for hydrocarbons analysis. It is based on the combination of the SMB technology with the Agilent 5975 GC-MS, forming a new and powerful GC-MS system. The GC eluting sample compounds are mixed with helium make up gas in a temperature programmable transfer line, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, and pass a fly-through, electric field free EI ion source where they are ionized by 70 eV electrons as vibrationally cold molecules (hence the name Cold EI). Ions originated from the beam molecules continue their straight flight, exit the ion source, are reflected at right angle towards the quadrupole MS, are mass analyzed and detected.

GC-MS with SMB enables the analysis of large wax compounds up to C74 via the use of short columns with high column flow rates to ensure their proper elution from the GC, as well as due to the features of fly-through ion source that does not cause any peak tailing of low volatility compounds. GC-MS with Cold EI (using SMB) provides trustworthy and largely enhanced molecular ions to all analytes as well as enhanced high mass fragments, for their improved identification. Isomer abundance analysis is enabled for obtaining unique and powerful hydrocarbon mixtures characterization method, that can be used for geochemical information, fuel and oil characterization and source location in oil spill and arson investigations.

The analysis of the following samples will be presented and described: polywax, oxygenates in wax, flesh flies wax, flower wax, beeswax on fruits and vegetables, jet engine oil, Jojoba oil, Nonoxynol-9 condom spermicide oil, motor oil freezing properties, triglycerides in biodiesel Diesel, fuel characterization, biodiesel in jet fuel and more.

Novel aspects:
GC-MS with supersonic molecular beam and cold-EI of vibrationally-cool molecules in fly-through ion source. Improved hydrocarbons identification and characterization with compounds extended range, enhanced molecular ion, novel isomer abundance analysis.
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 + C₂H₄O₂⁻ (m/z 280.9), RDX + C₇H₆CO₂⁻ (m/z 343.1). Analysis of RDX spiked with ammonium acetate also generated an adduct ion of RDX + C₂H₄O₂⁻ (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
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°C, 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
- 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
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 metabolite 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\textsuperscript{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.
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.
Determination of DNA adducts originating from methyleugenol using isotope-dilution UPLC-ESI-MS/MS

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Keywords: DNA adducts, UPLC-ESI-MS/MS, methyleugenol, MRM quantification

Methyleugenol (ME) is a secondary metabolite present in many herbal spices. Former observations indicate that hydroxylation followed by sulfation is an important bioactivation pathway of ME and other alkenylbenzenes leading to carcinogenicity in animals. In this context, DNA adducts of activated alkenylbenzenes have been detected in vitro and in vivo using the ³²P-postlabeling assay. This method cannot provide structural information of the detected adducts. Therefore, the aim of the present study was to characterize the chemical structures of ME-derived DNA adducts on the basis of MS-MS fragmentation patterns. Furthermore, we developed a sensitive quantification method using UPLC-ESI-MS/MS suitable for studying the bioactivation as well as adduct formation potential of ME in vitro and in animal models.

The UPLC-ESI-MS/MS method developed is based on the analytical determination of adducted 2'-deoxynucleosides using isotopic dilution analysis. For this purpose, stable-isotope labeled adduct standards of the ME-derived 2'-deoxyguanosine adduct and 2'-deoxyadenosine adduct were synthesized. Extracted DNA is enzymatically hydrolyzed into 2'-deoxynucleosides with addition of stable-isotope labeled compounds as internal standards. Afterwards the analytes are separated from digestion mixture ingredients by protein precipitation using ethanol. The 2'-deoxynucleoside adducts are chromatographically separated within 4 minutes. MRM data are acquired in the positive ion mode using three transitions for each adduct. All analyses are conducted with an ACQUITY UPLC (Waters) connected to a Xevo™ TQ MS (Waters).

By means of daughter scan experiments we were able to identify the structures of DNA adducts originated from ME, which were confirmed by NMR data. Consequently we generated MRM methods for these adducts. Therefore, we could identify and absolutely quantify adducted 2’-deoxyguanosine and 2’-deoxyadenosine in samples resulting from in vitro and in vivo experiments. The achieved sensitivity of this method is nearly one adduct per 10⁸ nucleosides.

In summary the new developed method represents a suitable and improved alternative for detection of ME-derived DNA adducts. Furthermore, ME-derived RNA adducts can be analyzed in the same UPLC run applying RNA adduct specific MS-MS transitions.

By means of this method we investigated the bioactivation pathway of ME via sulfation as well as the DNA adduct formation potential in vitro (1) and in vivo. Moreover, we even could demonstrate the presence of ME-derived DNA adducts in human tissues.


Novel aspects:

Development and application of UPLC-ESI-MS/MS method for quantification of DNA adducts originated from methyleugenol