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Ebook - Current Trends in Emerging Contaminant and POPs Analysis

Experienced environmental analysis experts share recent developments in emerging contaminant and POPs analysis

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This Ebook provides article summaries of lectures from today’s leading environmental researchers. It contains various approaches and techniques used in the analysis of POPs and emerging environmental contaminants

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Overview

Unknown organic contaminants in environmental samples

In the first article, Dr. Paul Jones, Associate Professor from the University of Saskatchewan, will discuss recent developments in ‘ultra-high’ resolution mass spectrometry systems that provide new opportunities for the discovery, analysis, and quantitation of small molecule environmental contaminants. Initial investigation of the occurrence of natural organobromine compounds in freshwater ecosystems, including a variety of both natural and anthropogenic organohalogens, was performed. The use of Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer, with ESI for organo-brominated and iodinated compounds in media as diverse as house dust and marine and freshwater sediments, is discussed. In addition, numerous novel brominated water disinfection by-products have been identified using similar techniques. The results of these studies, as well as the implications of the findings, are reviewed here.

Source water contaminant analysis

Equal to the importance of unknown contaminant identification is further investigation into known contaminants. Recently, interest in source water contamination has piqued due, in part, to the presence of Cyanotoxins. A noted increase in harmful algal blooms has been observed, exposing waters to toxic cyanobacterial metabolites. These compounds appear naturally in cyanobacterial blooms that have increased as a result of climatic changes and the eutrophication of water bodies caused mainly by agricultural wastes. In our second article, Dr. Audrey Roy-Lachapelle, Postdoctoral Fellow in Environmental Analytical Chemistry at the Université de Montréal, discusses the analysis of cyanotoxins using high resolution mass spectrometry. Using a Q Exactive Orbitrap mass spectrometer, the assessment of various cyanotoxins, through chemical derivatization and the study of fragmentation patterns in conjunction with the Thermo Scientific™ Mass Frontier™ 7.0 software, ultimately resulted in a method for total microcystin quantitation. The possible underestimation of microcystin levels in contaminated lakes, fish, and food supplements is reviewed.

Optimization for broad class contaminant analysis in environmental matrices

The diversity of current emerging contaminants includes compounds with highly diverse physical properties, as seen in the numerous microcystin compounds analyzed by Dr. Roy-Lachapelle. The complexity makes analyzing such classes of contaminants very challenging and often requires multiple analysis techniques, which is a very time consuming process. While many labs utilize separate methodologies for each compound class, current technology exists to allow for casting a wider net when it comes to optimizing analysis. In the third article, David Schiessel of Babcock Laboratories, California, examines instrument and sample prep techniques in an online SPE approach to analyzing broad classes of emergent contaminants in various matrices. Compound classes that are discussed include pharmaceuticals, personal care products, hormones, perfluorinated compounds, and selected pesticides, as well as the importance of mass spectrometry parameter optimization to dramatically increase one’s ability to discriminate analytes in difficult environmental matrices.

Urban wastewater treatment contaminants

The prevalence of new contaminants, and the large breadth of classes that encompass contaminants' subsequent evolution of wastewater quality regulations, has followed. To meet new regulations, wastewater treatment facilities are exploring improvement of treatment strategies to ensure removal of contaminants. Research efforts are leading the way to determine what is needed at this stage of development and the implementation of technologies to get the full benefits from these upgrades. Special attention has been given to CECs and their transformation products (TPs) formed during treatment. In our fourth article, Dr. Viviane Yargeau, from McGill University in Montreal, discusses a program for the development of a more global evaluation of ozone-based technologies for disinfection of wastewater to address the CECs issue. Topics discussed include the identification and structural elucidation of TPs using LC-HRMS, including the analysis of hormones, antibiotics and illicit drugs. Also discussed is the use of Thermo Scientific™ SIEVE™ differential analysis software, along with a "Control Compare Trend" experiment in order to monitor the various signals as a function of O3 dosage and narrow candidate TP compounds.

Industry discharge impact and fate analysis

In addition to urban wastewater treatment, industrial discharge is another area of major concern for environmental research. Hussain Abdulla of Texas A&M University - Corpus Christi, investigates the fate and impact of organic compounds from the release of oilfield-produced water (a.k.a petroleum brine) in the Texas Gulf Coast. It is estimated that over 32 million gallons of petroleum brine were discharged daily to Texas tidal waters. Using untargeted analyses capabilities of a state-of-the-art, Thermo Scientific™ UPLC-Orbitrap Fusion™ Tribrid™ Mass Spectrometer system in order to identify dissolved organic compounds in Nueces Bay, Texas, of 15 surface water stations, revealed the existence of around 4,000 organic compounds in each station. Also noted at these sites was a high abundance of organophosphorus compounds (over 800 compounds per sample) that fell outside the natural phospholipids and phosphopeptides region. Further discussion reveals the proposed interaction of these compounds with organophosphorus insecticides and herbicide artifacts used on the neighboring agricultural area, and now present but hidden, in the bay sediment.

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Investigating the Occurrence of Organohalogens in the Environment Using High Resolution Accurate Mass Spectrometry

Authors: Dr. Paul Jones, Associate Professor, Dr. Hui Peng, and Dr. John Giesy, Professor and Canada Research Chair in Environmental Toxicology, University of Saskatchewan, Canada

Some organohalogens are environmental pollutants known to be highly persistent, bioaccumulative, and toxic. Industrial and pharmaceutical incorporation of organohalogens has become a significant source of human exposure. Polyvinyl chlorides (PVCs), flame retardants, solvents, additives, and some drugs contain a form of organohalogen. Given their pervasiveness in consumer products, studies regarding their effect on human health are paramount to safety regulations for chemical use. Focus on untargeted screening and identification of a variety of organohalogen compounds is an important step toward ensuring consumer safety of products.

Organohalogens in the environment

Organohalogen compounds are natural and synthetic chemicals containing one or more halogen atoms (fluorine, chlorine, bromine or iodine) combined with carbon and other elements. Work on the range of both anthropogenic and natural organohalogens in the environment, where they are coming from, and their potential toxicity, provides a basis for standards and regulations around their use. A study of the relative contribution of identified organobromine (OBr) concentrations in the livers of polar bear, albatross, and tuna from remote marine locations indicated accumulation of a variety of both known and unknown brominated compounds[1]. Results from studies like these suggest that anthropogenic compounds can leach into the environment, and that there is an as yet undefined variety of naturally occurring organohalogen compounds.

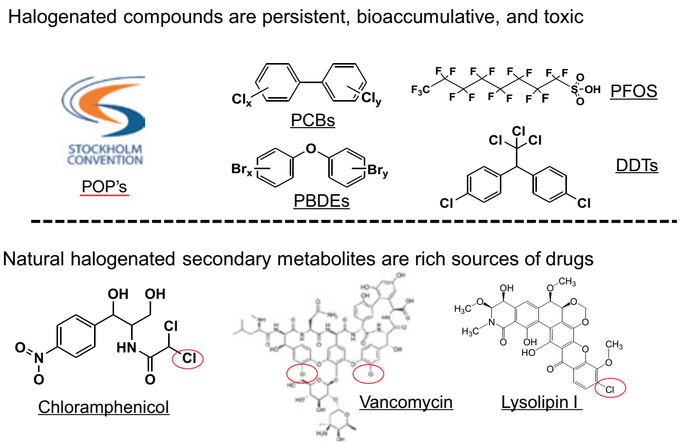


Figure 1. A sampling of known organohalogens

Since the vast majority of natural organohalogens are unknown, untargeted screening permits detection and identification of various organohalogens and can assist in estimating accumulation potential in animals and humans. High resolution liquid-chromatography-mass spectrometry (HR LC-MS) is a fitting technique for the untargeted screening and identification of organohalogens based on the instrument's ability to resolve masses and identify ions to a level of precision that can permit chemical formula and structural elucidation.

DIPIC-Frag method development

A novel LC-MS method using data-independent precursor isolation and characteristic fragment identification (DIPIC-Frag), identifies organohalogens based on the presence of halogen ions as the characteristic fragment[2]. Using both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) allows generation of parent ions with minimal fragmentation while higher-energy collisional dissociation (HCD) dissociates and characterizes the parent ions based on the presence of the halogen fragment. The method described is run on the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer (Figure 2). The instrument offers high resolution (mass accuracy <2 ppm), high sensitivity, quadrupole isolation of precursor ions, and the ability to run multiple acquisition modes simultaneously.

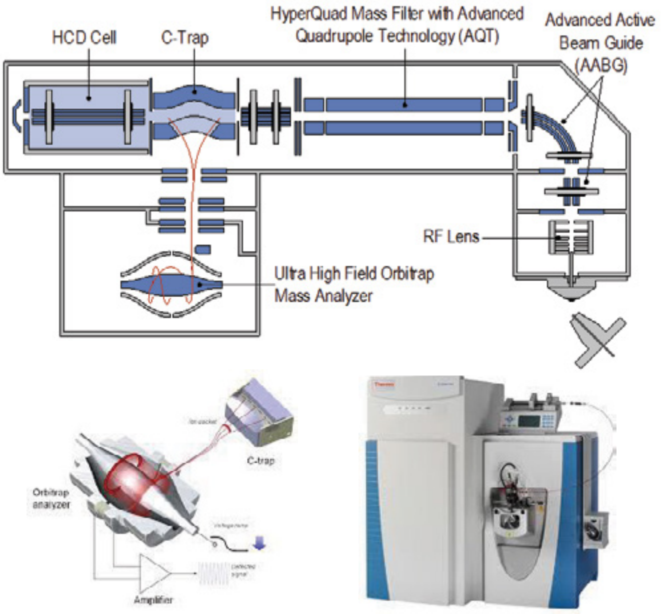


Figure 2. The Thermo Scientific Q Exactive mass spectrometer

To find characteristic halogen ions from samples with multiple compounds, full scans are run concurrently with a series of data-independent MS/MS acquisitions (DIA) windows to spread the compounds into smaller groups for separation and identification of individual compounds. Each DIA window series comprises of 10 separate runs to cover a large mass range from *m/z* 100 to *m/z* 1000.

The resulting datasets are analysed with an R package for automatic data organization into interpretable units (Figure 3) such as ion fragment chromatograms, isotope analysis to estimate precursor alignment, elution profiles to confirm ion mass, and MS/MS patterns to extract structures and predict molecular formulas.

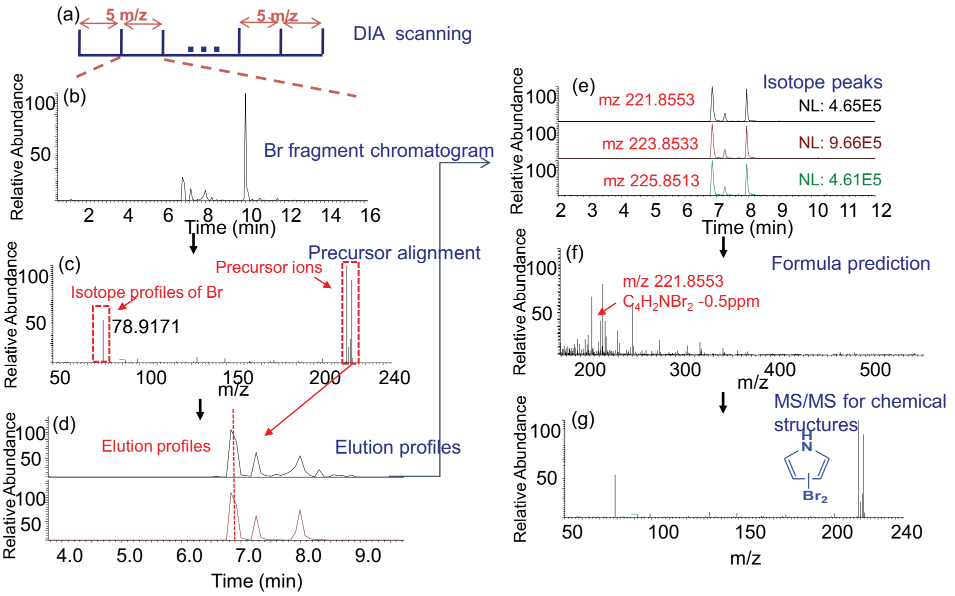


Figure 3. The datasets obtained

Method validation was performed on sediment samples from Lake Michigan[3]. The DIPIC-Frag method isolated 2,520 organobromine peaks total, averaging 15 OBrs per DIA window. The study analyzed relative intensity since no standards are currently available for the identification of many ‘unknown’ organohalogen compounds. Initial results identified carbazoles, polybrominated diphenyl ethers (PBDEs) and brominated fatty acids (Figure 4). Carbazoles were present in unexpectedly high amounts and were further analyzed as a contaminant of interest with toxic potential.

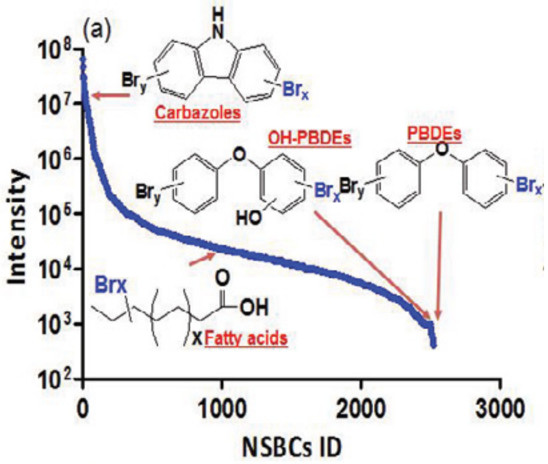


Figure 4. Initial organohalogen compounds identified

Identification of organobromines in house dust using DIPIC-Frag untargeted screening

House dust is surprisingly rich in contaminants from furniture, carpeting, clothing, toys, and other items. House dust has become a noteworthy route for human exposure of organohalogen contaminants, especially for children, making it an ideal source for synthetic chemical screening.

The DIPIC-Frag method was performed on house dust samples, identifying more than 500 complex and chemically diverse compounds. Brominated flame retardants (BFRs) are one of the most common brominated compounds in house dust, but BFR concentrations were 100-fold lower than those of the measured total bromine concentration, indicating the existence of unknown brominated compounds (see Group III, Figure 5).

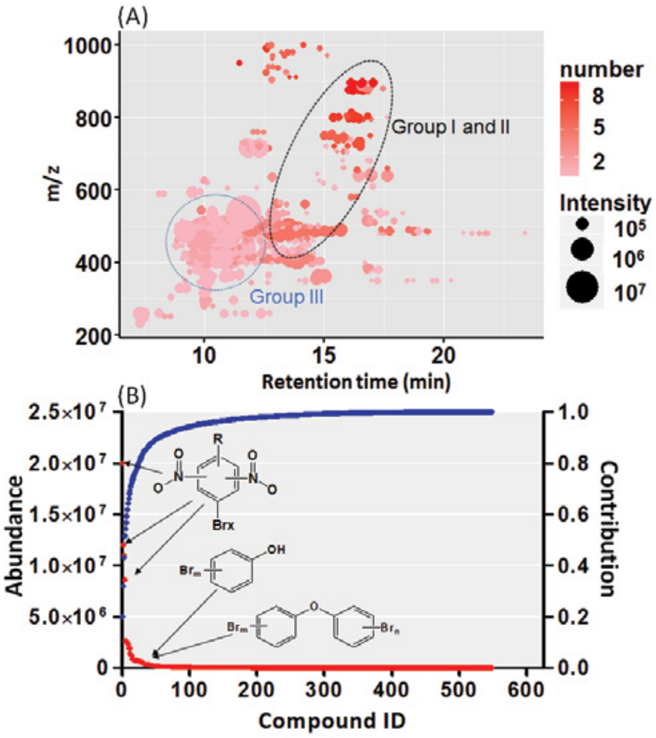
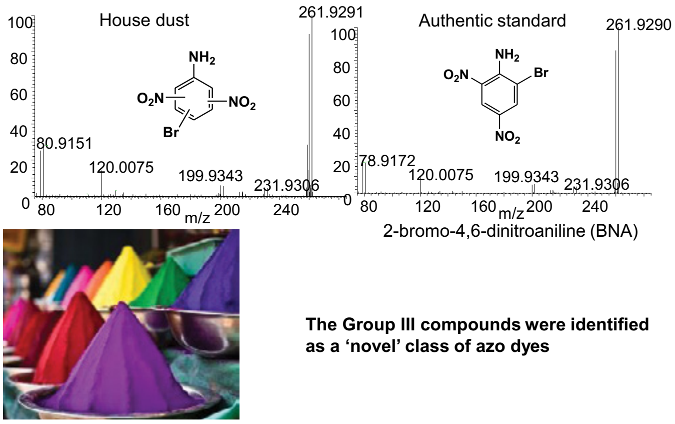


Figure 5. Analysis of house dust samples

The largest class of compounds was identified to be a 'novel' class of azo dyes, nitrogen-containing compounds with small bromine atom numbers (Figure 6)[4]. The detected levels of these compounds in dust samples, particularly 2-bromo-4,6-dinitroaniline (BNA) measured at 1.7 µg/g. BNA has been shown to induce mutagenicity at concentrations of 1.25 µg/mL, indicating the potential health risks depending on exposure scenarios.



The Group III compounds were identified as a 'novel' class of azo dyes

Figure 6. Identification of the largest class of compounds

Identification of novel brominated disinfection by-products of concern in drinking water using DIPIC-Frag untargeted screening

DIPIC-Frag was also used to detect brominated compounds that form as by-products of the water disinfection process, which sterilizes water to make it safe for human consumption. When natural organic matter and inorganic halogen ions in water react with a chlorine-based disinfectant, various unknown brominated disinfection by-products (DBPs) are created. These compounds could potentially have toxic effects if consumed. This is generally not an issue for water treatment plants since most facilities typically remove organic matter from the water before disinfection treatment. However, by analyzing water treated at the intake source, standards can be tested for all facilities to consider what creates the safest treatment methods.

DIPIC-Frag was performed on a series of treated water samples to generate a library of various organobromine and organoiodide DBPs, employing different solid phase extraction (SPE) techniques, separation columns, ionization methods, and SPE pHs (Figure 7). 1,500 compounds were identified that are generated during water treatment. Dominant compounds were polar and water-soluble, including the known haloacetic acids and also other unknown DBPs.

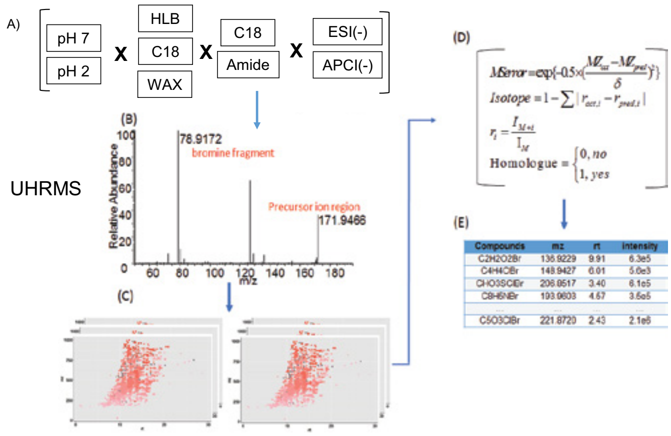


Figure 7. DIPIC-Frag results

The top 50 brominated DBPs contributed to 35.6 percent of the total organobromine mass. Structural prediction of 41 of the 50 compounds was achieved using high-resolution accurate mass (HRAM) analysis and MS/MS data. These compounds included aromatic acids or phenols, nitrogen or sulfur containing heteroatomic compounds, and high concentrations of sulfonic acids (Figure 8). With over 700 compounds established in the library and most not previously reported in drinking water, the DIPIC-Frag method displayed superior precision for compound identification.

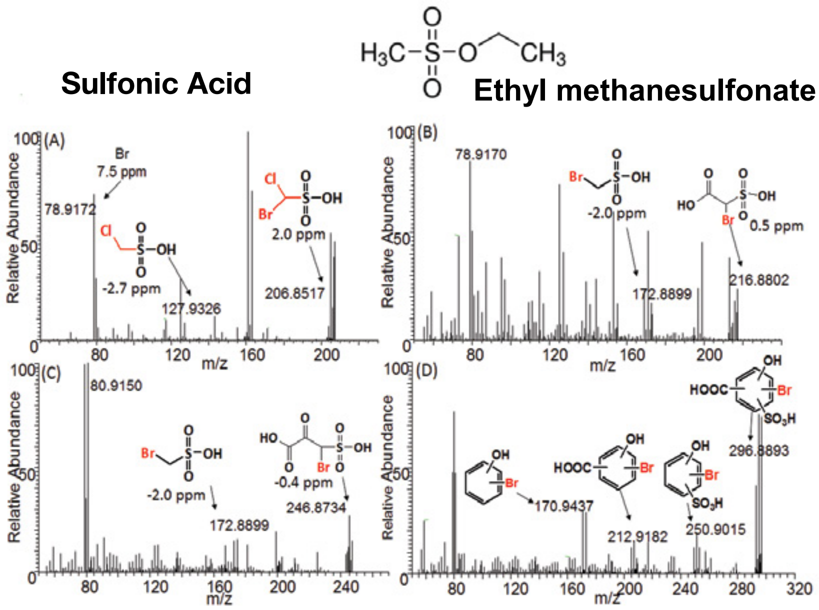


Figure 8. Identified compounds

Conclusion

These studies validate the application of the DIPIC-Frag method using the Q Exactive mass spectrometer to establish comprehensive libraries based on HRAM analysis and MS/MS datasets. Defining organohalogen contaminants in the environment provides valuable knowledge about the type and origin of contamination and allows for the development of standards and regulations. Initial compound identification also triggers ongoing studies of compound toxicology for high abundance chemicals.

For more information, watch the video, 'Investigating the Occurrence of Organohalogens in the Environment', presented by Dr. Paul Jones - University of Saskatchewan

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ADDITIONAL RESOURCES

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Advantages of High-Resolution Mass Spectrometry in Environmental Analysis

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Unknown Screening of Environmental Contaminants

Articles

Screening of lipophilic marine toxins in shellfish and algae: Development of a library using liquid chromatography coupled to Orbitrap mass spectrometry

Identification of the unknown transformation products derived from clarithromycin and carbamazepine using liquid chromatography/high – resolution mass spectrometry

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Determination of Cyanotoxins Using High Resolution Accurate Mass Spectrometry

Author: Dr. Audrey Roy-Lachapelle, Postdoctoral Fellow in Environmental Analytical Chemistry, Université de Montréal

Cyanobacteria, also known as blue-green algae, proliferate naturally in lakes, rivers, and streams given adequate amounts of sunlight, heat and nutrients. Recent observations of frequent cyanobacterial overgrowth into large algal blooms are most likely due to increased nutrient run-off and fluctuations in water temperature due to climate change and intensive agriculture activities. Cyanobacterial blooms present major concern to aquatic ecosystems and human health due to their production of cyanotoxins. The use of contaminated surface water as source influent for drinking water treatment plants increases exposure risk. Increased emphasis has been placed on water treatment plants to monitor for the presence of cyanotoxins and prevent contamination through the removal of these potentially dangerous toxins.

Emerging threat of cyanotoxins

Prediction of whether or not cyanobacteria produce cyanotoxins is next to impossible. There are many toxic and non-toxic varieties of cyanobacteria, with toxic species making up an estimated 40 percent of bacteria in algal blooms (seen in figure 1). Given that toxic cyanobacteria may or may not produce cyanotoxins, depending on their growth conditions and environment, it is challenging to know when to treat water for toxins and when it is unnecessary. As a result, all water containing cyanobacteria must be treated for toxins.

Figure 1. Toxic algal blooms

Most guidelines for cyanotoxin concentrations in drinking or recreational water follow the World Health Organization (WHO) concentration recommendation of 1.0 μg/L and only include the most commonly known cyanotoxins. A preliminary test on drinking water treatment detected cyanotoxin levels in untreated water of 119 μg/L MC/L, with treated drinking water measured at 2.5 μg/L MC/L. Based on the US Environmental Protection Agency's regulated child protection guidelines of 0.3 μg/L MC/L, current water quality might not meet recommended levels for safe consumption.

Analytical method development faces several challenges due to the diversity of toxins, their congeners, and their various physicochemical properties. With such a wide variety of toxins produced, much work is needed to ensure confident detection and identification of each compound.

Determining β-N-methylamino-L-alanine in lake water using high-resolution mass spectrometry

Some harmful algal blooms contain specific alkaloid cyanotoxins such as the nonproteinogenic amino acid β-N-methylamino-L-alanine (BMAA), which currently has no regulation or monitoring procedures but is widely produced by cyanobacteria. A new analytical method was developed for detection of BMAA and two of its conformation isomers, 2,4-diaminobutyric acid (DAB) and N-(2-aminoethyl) glycine (AEG), as well as three alkaloid cyanotoxins, anatoxin-a (ANA-a), cylindrospermopsin (CYN), and saxitoxin (STX).

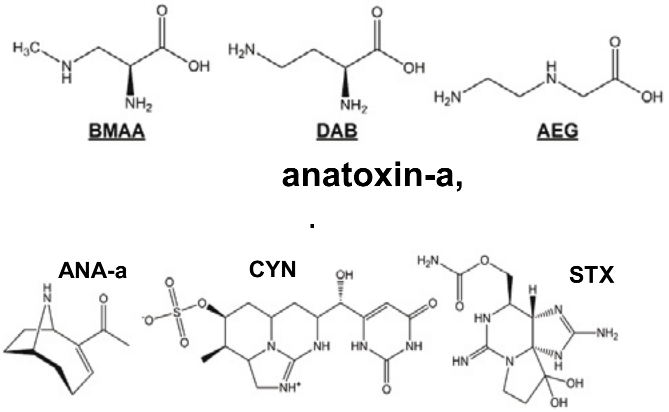

Figure 2. Chemical structures of tested compounds

The method uses chemical derivatization with dansyl chloride (DNS) for easier separation, followed by ultra-high-performance liquid chromatography coupled with heated electrospray ionization and the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™

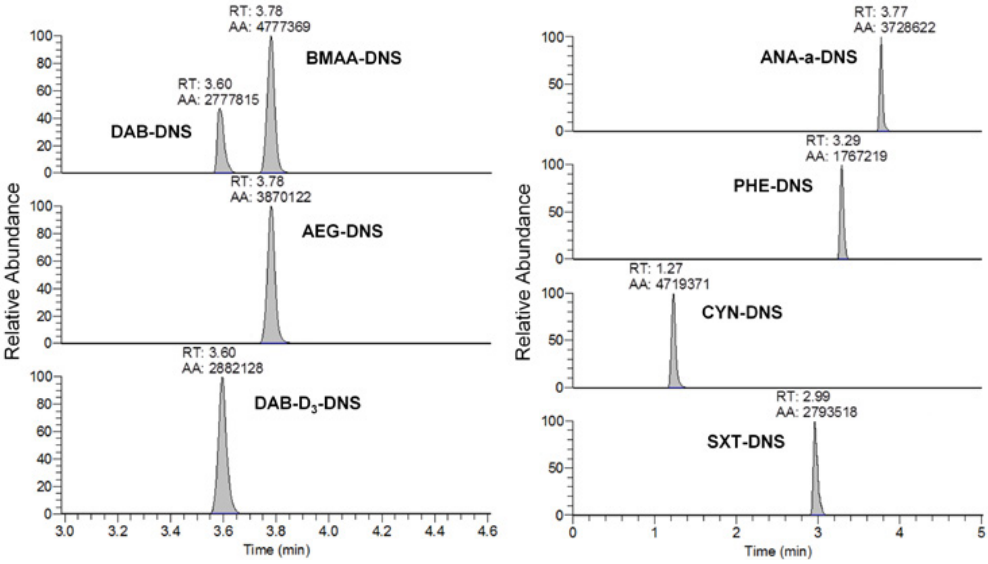

Figure 3. Chromatograms of DNS derivatives

mass spectrometer (UHPLC-HESI-HRMS) for highly selective fragmentation and exact mass detection to reduce isobaric interference. Fragment structures were suggested using Thermo Scientific™ Mass Frontier™ 7.0 software since BMAA and its isomers could not be separated by other techniques.

Method validation demonstrated linearity from 0.009 and 0.3 µg/L with low limits of detection. Results reveal the presence of BMAA in lake water, indicating that monitoring and detection protocols need to include these cyanotoxins during water treatment.

High-resolution accurate mass detection of Anatoxin-a

Anatoxin-a (ANA-a) is a known neurotoxin that mimics the effects of the neurotransmitter acetylcholine, causing respiratory complications or paralysis in exposed subjects. While ANA-a is under regulatory control, detection methods need improved sensitivity and selectivity to reduce possible false positives from isobaric interference of phenylalanine and to achieve lower detection limits.

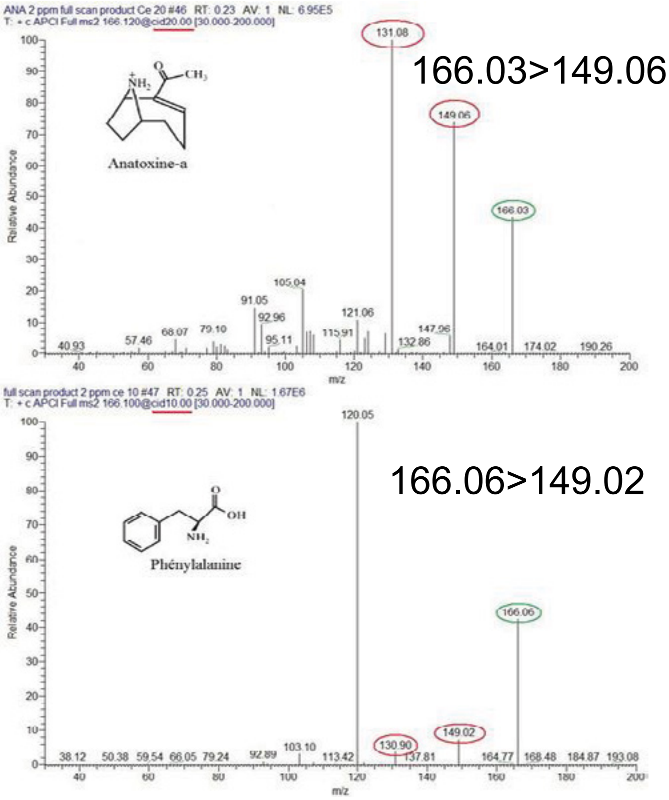

Figure 4. Anatoxin-a and phenylalanine

Employing laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI) coupled to the Q Exactive mass spectrometer created an LDTD-APCI-HRMS combination with parallel reaction monitoring (PRM) that allowed for an ultra-fast analysis time and differentiation of ANA-a from phenylalanine signals. Method validation on eight different water samples successfully revealed the presence of ANA-a at levels of 0.01-0.2 µg/L in all samples tested.

Analysis of total concentrations of microcystins in fish

Microcystins (MCs) are cyanobacterial toxins with 235 variants identified to date, yet only 15 regulatory standards are in place. These toxins have the capacity to covalently bind to tissue allowing their bioaccumulation in animals and can thus be subjected to biomagnification.

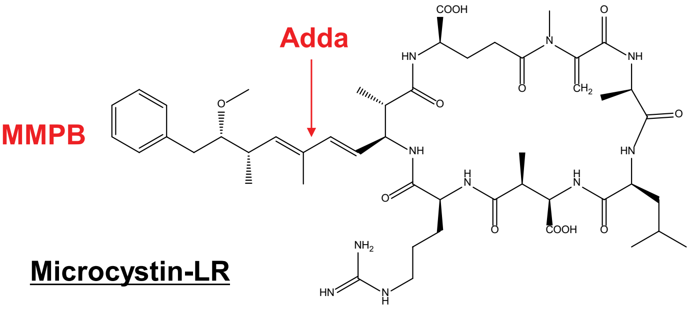

Figure 5. MMPB moiety

An approach for cell-bound and free microcystin analysis was developed using sodium hydroxide as a digestion agent and Lemieux oxidation to target the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) moiety, common to all microcystin congeners. LDTD-APCI-HRMS was applied to fish tissue samples from cyanobacteria-contaminated lakes and reported microcystin concentrations in all samples ranging from 2.9 to 13.2 µg/kg. However, when using standards to test for microcystin presence, only one of 25 samples was positive. This indicates that there are more microcystin congeners than originally expected, and the described novel approach is able to detect these additional congeners.

Detection of cyanotoxins in cyanobacterial dietary supplements

Cyanotoxin contamination in dietary supplements is a known issue in products with few quality control standards. Although supplements are believed to benefit overall health, cultivation methods of non-toxic cyanobacteria allow contamination by toxic species present in the natural environment. To test for toxic levels of contamination, the presence of total microcystins, including seven individual microcystins, anatoxin-a, dihydroanatoxin-a, epoxyanatoxin-a, cylindrospermopsin, saxitoxin, and β-methylamino-l-alanine was investigated in 18 different commercially available products containing *Spirulina* or *Aphanizomenon flos-aquae*.

Total microcystin analysis was accomplished using Lemieux oxidation and chemical derivatization using dansyl chloride for the simultaneous analysis of cylindrospermopsin, saxitoxin, and β-methylamino-L-alanine. LDTD-APCI-HRMS enabled high performance detection and quantitation of products, resulting in identification of eight samples containing cyanotoxins at levels exceeding the recommended daily intake values, some by up to almost 700 percent. Detecting cyanotoxins in these algal dietary supplements reinforces the need for better quality control and better consumer awareness regarding the potential risks associated with their consumption.

Conclusion

With the goal of accurate and more specific determination of cyanotoxins in the environment, UHPLC-HESI-HRMS and LDTD-APCI-HRMS provide successful identification of known and unknown cyanotoxins in water and tissue samples. When comparing results using these methods with current standards, this new approach detects more cyanotoxins at much lower detection limits, allowing identification of new congeners and separation from potential interferences. Future development includes coupling online solid-phase extraction to UHPLC and HRMS for larger scale monitoring efficiency. Detection of much higher levels of cyanotoxins than previously reported emphasizes the need for improved regulations and standards development in addition to more in depth toxin analysis to ensure comprehensive water treatment.

For more information, watch the video New Strategies for the Determination of Cyanotoxins Using High-Resolution Mass Spectrometry, presented by Dr. Audrey Roy-Lachapelle, Université de Montréal

References:

https://www.epa.gov/sites/production/files/2014-08/documents/cyanobacteria_factsheet.pdf

ADDITIONAL RESOURCES

Application Notes

Quantitation of cyanotoxins in drinking water according to EPA 544 guidelines

Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545

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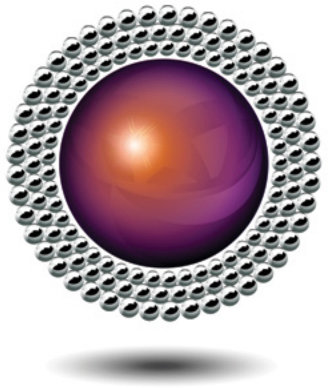

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An Online SPE Approach to Analyzing Broad Classes of Emerging Contaminants in Various Matrices

Author: David Schiessel - Babcock Laboratories, California

The analysis of emerging contaminants has become more challenging due to an increase in the number of, and diversity of, analytes detected in the environment. These analytes comprise a diverse group of contaminants with a wide range of physical properties including personal care products, pharmaceuticals, and perfluorinated compounds. This diversity makes the development of comprehensive analysis techniques challenging. Current methods encompass several different targeted assays depending on the chemical class that is being analyzed. However, technological developments have been made that enable users to design improved and less complicated processes. Applying new developments in online solid phase extraction (SPE) to isolate chemicals prior to liquid chromatography tandem mass spectrometry (LC-MS/MS) allows the simultaneous extraction of multiple and diverse contaminant compounds and their subsequent analysis and identification.

Challenges with current methods and new developments

Given the wide range of chemicals that must be tested and identified in the environment, from water sources to accumulation in soil, safety organizations such as the US Environmental Protection agency (EPA) have developed a series of methods for use across a variety of compound analysis requirements. For example, EPA Method 1694 provides a standard for the detection of pharmaceuticals and personal care products in water, soil, sediments, and biosolids using high performance liquid chromatography HPLC-MS/MS. This method allows for multiple eluent conditions for various classes of compounds across four analyte types. However, current techniques may not be appropriate for newer analytes, making this method less robust for diverse analyte detection.

New developments create the opportunity to expand the range of contaminants that can be analyzed under one method. Improved electrospray ionization (ESI) with new eluent systems and the newest polymeric classes of SPE sorbents are initiating a move to online SPE techniques resulting in decreased analysis time and the ability to bypass sample preparation all together. In fact, a new method, EPA 543 applies online SPE to the determination of organic chemicals in drinking water coupled with LC-MS/MS. Further improvements in instrumentation such as fast ESI polarity switching and high duty cycles with smaller full-width at half maximum (FWHM) offer increased specificity and higher quality signal to noise ratios.

Online SPE approach to emerging contaminant analysis

Online SPE can be defined as automated solid phase extraction performed at the instrument with the capability to load a filtered or unfiltered sample. This eliminates the need for time-consuming sample preparation and permits the use of an entire sample without effecting recovery, allowing raw samples to be easily analyzed. Specialized short liquid chromatography columns are used (20-50mm) with a large particle size to allow loading of aqueous samples at a higher flow and decreasing sample dwell in the columns. In addition, elution is performed by backflushing the SPE sorbent for further selectivity of peak signals. Other varieties of the online SPE technique include turbulent flow for tougher matrices such as blood and urine.

There are several considerations that can help to optimize online SPE to varying groups of chemicals. A key step to the method regardless of the chemicals being analyzed is washing the SPE with nanopure water to remove preservatives or matrix salts (usually chloride and sulfate) that can interfere with compound identification and quantitation stemming from ion suppression. Faster gradients also assist in eluting the analytes off of the column, particularly if there is any issue with “sticky” analytes associated with the presence of highly polar moieties or hydrophobicity.

Given the broad range of chemical classes that need to be analyzed, optimization of monitoring parameters for specific analyte classes can help generate robust targeted experiments. With pharmaceutical and personal care product (PPCP) analysis, for example, transitions are optimized at a lower collision-induced dissociation (CID) pressure, especially when some optimal collision energies are less than 10 eV. For sample preparation using polymeric SDVB (Strata-X) online SPE helps to increase retention of highly polar analytes such as acetaminophen or iopromide. Additionally, online SPE devices can be “stacked” in a similar fashion to offline SPE. In this technique, a polymeric SDVB is connected on the back end of a C18 SPE device. Analytes that would normally break through a C18 SPE device are captured by the polymeric SDVB; this includes sucralose and iopromide. Specific analysis conditions are shown below, and sample chromatograms are shown in Figure 1.

PPCP analysis conditions

- Sample: prepreserved with sodium azide and ascorbic acid. Add 3 drops formic acid per 40 mL prior to analysis
- Mobile phase: 300 µL/min 5 mM Ammonium Hydroxide with 10-80% Methanol
- Online SPE media: Thermo Scientific™ Hypersil™ C18 and Strata-X (polymeric) for Sucralose/Iopromide (can use HyperSep Retain PEP7)
- MS parameters: Thermo Scientific™ TSQ Quantum Ultra™ Triple Quadrupole LC - Mass Spectrometer. Vap/Cap Temps at 250 ˚C, Sheath = 35, Aud = 15, CID = 1.0 mTorr Argon, Q1 = 0.4 FWHM
- Quantitation method is strict isotope dilution

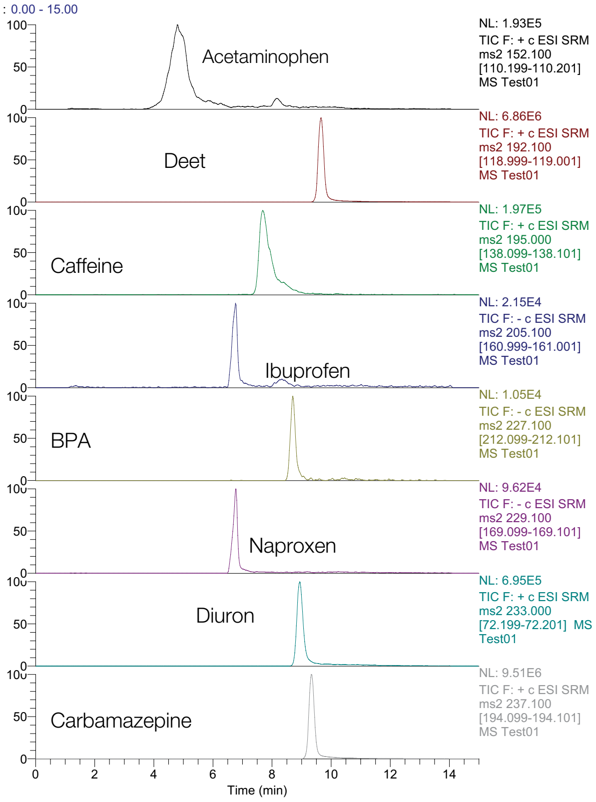

Figure 1. A sampling of PPCP product chromatograms

Another chemical class, perfluorinated compounds, has slightly different optimal method adjustments. The wash step for these compounds is critical because the sample contains 5 g/L of Trizma as a preservative. The most robust media for this class of compounds is a weak anion exchanger, especially if PFBA is to be determined. Because of this mechanism, a high pH is required to elute analytes off of the sorbent using the mobile phase. But, high pH is usually detrimental to most silica based HPLC columns. To resolve this, specific columns that are stable at a high pH (approx pH of 11) can be used. Specific PFC analysis conditions are:

- Sample: Prepreserved with 5 g/L Trizma
- Mobile Phase: 300 μL/min 0.24–0.08% Ammonium Hydroxide with 40–80% Methanol
- Online SPE media: Strata-X-AW (mixed mode weak anion) works especially well for PFBA/PFPeA
- LC Column: C18 EVO (Stable at high pH)
- MS parameters: TSQ Quantum Ultra MS. Vap/Cap Temps at 250/150 C, Sheath = 40, Aux = 40, CID = 1.0 mTorr Argon, Q1 = 0.4 FWHM
- Quantitation method is internal standard by compound class

Method limitations and solutions

Matrix issues are a recurring obstacle across any sample analysis. Depending on the matrix that a sample is collected in, method adjustments must be made. Clean matrices such as drinking water can be directly analyzed using online SPE followed by LC-MS/MS. However, many matrices tend to be high in chloride and sulfate salts, causing amplified background noise and/or ion suppression. By using a Thermo Scientific™ Dionex™ OnGuard™ cartridge, the sample passes through the cartridge while the salts remain, resulting in an average 90 percent reduction in salt contamination. After loading samples, a wash step and filtering with glass fiber can be used to further clean the sample. Of course, any filter used must be verified to be free of target analytes. Some glass fiber filters have a contaminant that is an isobaric interference to the common PFOS transition often used (499 -> 80).

Another way to improve matrix discrimination is by carefully choosing an optimal FWHM. A smaller FWHM can eliminate background and help distinguish peaks since different background interferences can originate from different chemical sources. However, decreasing the FWHM too much can lead to diminished peak signals stemming from mass instability. Optimizing the peak signal by testing a range of FWHM values can help maintain the signal to noise ratio at its optimal value. For example, testing FWHM values of 0.7, 0.5, 0.2, and 0.04 Da for the analyte cyfluthrin demonstrates high background noise at 0.7 Da and diminished peak size at 0.04, making 0.2–0.4 Da the ideal range, as shown in Figure 2.

Choosing particularly low values for FWHM, however, requires specific attention to mass calibration and stability. Mass calibration using phosphoric acid clusters can improve calibration stability because 9-10 masses are used during mass calibration instead of the standard 3 masses using 1,3,6-PolyTyrosine. Mass stability is best examined with a FWHM range of 0.06-0.7 Da on a mass spectrometer using profile mode to examine each mass as continuous data, with the data points defining the curve corresponding to the signal intensities at each particular *m/z* value. This allows inspection of the peak apex and whether or not the reported mass by the MS data is within the desired limit based on expected *m/z* values. At high FWHM values, the mass stability has less of an effect on centroiding shows that the true masses rest in the center of each peak. If the FWHM is too low, the mass stability may cause a measured mass to fall

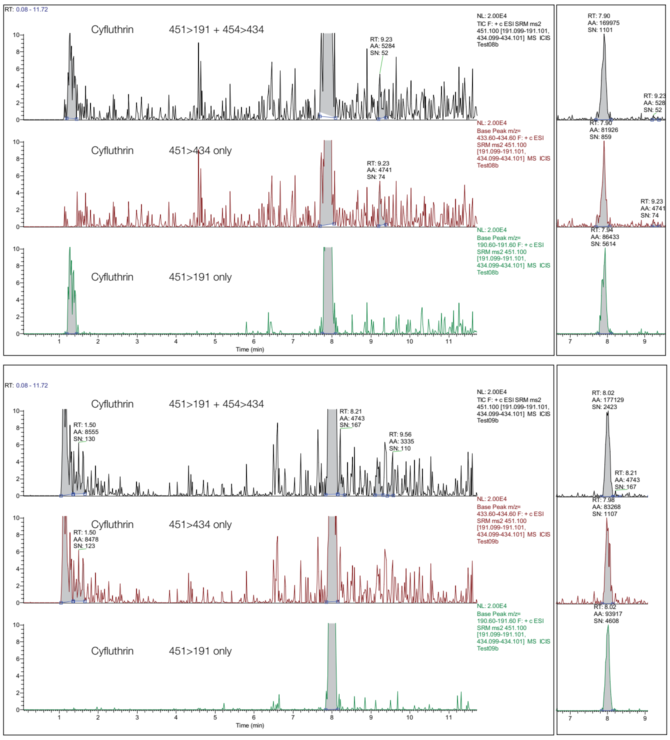

Figure 2. Effects of FWHM for cyfluthrin at 0.7 Da and 0.2 Da.

outside of the FWHM window, resulting in erroneous mass value determinations and poor precision of data (Figure 3).

Conclusion

Online SPE is a robust approach to multi-analyte testing across a broad and growing selection of analytes of interest, allowing a wide range of contaminant classes to be analyzed simply by optimizing the online SPE method prior to LC-MS/MS. The online SPE approach provides the ability to eliminate sample preparation and decrease time spent on analysis. Online SPE may also be adapted to soil analysis if aqueous extraction is used, providing an even broader analytical relevance. Targeted optimization, as described for various analyte classes, makes online SPE a viable approach to contaminant analysis.

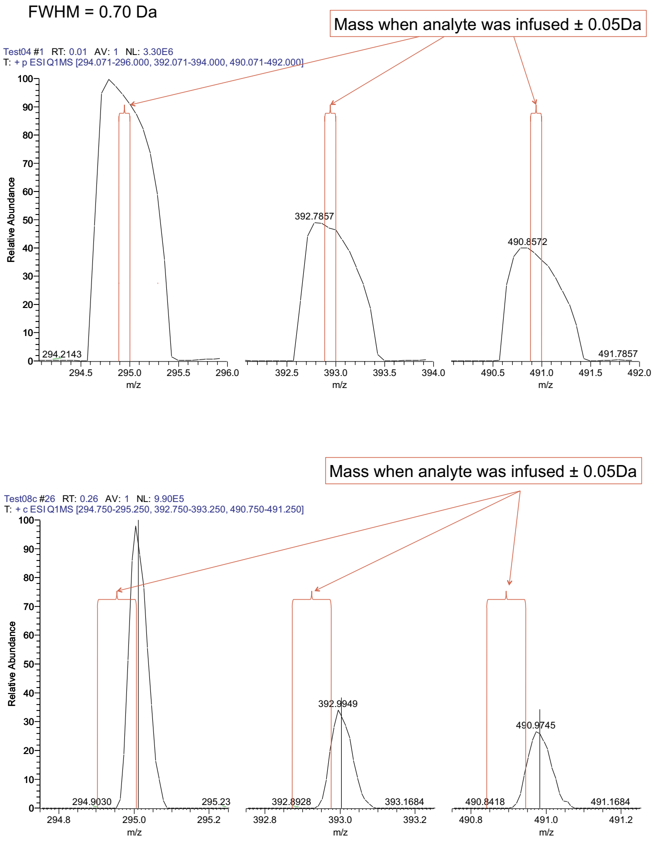

Figure 3. FWHM analysis at 0.7 Da and 0.06 Da on a MS in profile mode

For more information, watch the video, "An online SPE Approach to Analyzing Broad Classes of Emergent Contaminants in Various Matrices", presented by David Schiessel - Babcock Laboratories

ADDITIONAL RESOURCES

Brochure

EQuan MAX Plus™ System for water testing

Application Notes

Analysis of Basic and Acidic Pharmaceutical Products in Drinking Water Using Online Sample Preparation and LC-MS/MS

Analysis of Chloroacetanilde and Other Acetamide Hermacide Degrades in Drinking Water by LC-MS/MS

Analysis of Regulated Pesticides in Drinking Water Using Accela and EQuan


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LC-MS as a Tool for Optimizing Wastewater Treatment

Author: Dr. Viviane Yargeau, Department of Chemical Engineering & McGill School of Environment, McGill University, Montreal

While Contaminants of Emerging Concern (CECs) in water sources are routinely detected, their transformation products (TPs), generated during water treatment and from naturally occurring reactions in water, are surfacing as a more recent challenge in water safety. TPs can have comparable or even greater toxicity risks than their parent contaminant and can go undetected in water sources since they are unknown or prioritized differently than CECs. Detection and identification methods for TPs require unparalleled specificity in order to elucidate new structures that are similar to known contaminants but need to be separated as individual compounds.

The challenge of unknown contaminants

Certain chemicals, including medicines, personal hygiene products and various chemical additives make their way into the environment through wastewater treatment plants, runoff from agricultural and urban land surfaces, and septic systems. These compounds are known as CECs and represent a threat to water safety and public health from both industrial production and domestic use.

CECs are a known group of contaminants for which safety organizations, such as the U.S. Environmental Protection Agency (EPA) have set concentration level recommendations and guidelines. CECs such as estrone (a hormone), levofloxacin (an antibiotic), and MDMA (the illicit drug, ecstasy), for example, can have severe adverse effects on aquatic life and human health and so must be regulated. These contaminants can also create TPs that are not under regulatory control, primarily because there are not robust methods for identification of these intermediate compounds.

TPs have been detected at low levels in wastewater treatment plants and in the environment as a result of a variety of factors such as hydrolysis, photolysis, oxidation and microbial metabolism acting on their parent compounds. These products can be a cause for concern since they can be biologically active and resistant to further degradation. Disinfection treatments such as ozonation, which oxidizes organic and inorganic contaminants based on a reaction with ozone, or O3, remove parent compounds, but are not necessarily proven to remove TPs. Increased focus has been given to the nature and safety of these transformation products through toxicity testing and identification of specific intermediates.

Determination of transformation products from estrone using LC-HRAM

In an effort to develop a faster and more reliable method for the identification and elucidation of TPs from water samples, a combinatory approach was taken with liquid chromatography-high resolution Accurate Mass spectrometry (LC-HRAM) followed by extensive data mining and interpretation. Combining these methods allows standardized identification and quick elucidation of structural variants.

A simple batch experiment was performed to validate this approach. A model compound, estrone (E1), was mixed with ozone stock solution across a series of E1:O3 molar ratios ranging from 1:0 to 1:8 with estrone concentrations of 0.05-2 mg/L to monitor bioactivity as a function of ozone dose using the yeast estrogen screening (YES) bioassay. Estrogens and other steroid hormones are CECs that are typically removed during wastewater treatment, yet estrone has been reported in water sources and can act as an endocrine disruptor in the body. Additionally, E1 can be removed using ozonation treatment but is known to create TPs left behind in treated water. In removing estrone by ozonation, estrogenic activity should decrease proportionately.

Initial and residual E1 concentrations were measured by LC-HRAM using a Thermo Scientific™ Accela™ LC system coupled to a hybrid mass spectrometer consisting in a linear ion trap interfaced to a Thermo Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap mass spectrometer. Estrogenic activity showed a slight initial increase at lower molar ratio doses (2:1 and 1:1) followed by a decrease at higher molar ratios. While the decrease at higher ozone ratios indicated successful ozonation, the initial increase in activity questions whether current treatments are enough to reach the targeted level of estrone and TP removal.

Differential analysis of transformation products

The sample series was analyzed with an LTQ Orbitrap XL mass spectrometer in both APCI(+) and APCI(-), and acquisition files were processed with Thermo Scientific™ SIEVE™ software to look for transformation products. Use of SIEVE software allows extraction of signals from the acquisition files using a three coordinate read of retention time, *m/z* value, and intensity, resulting in a 3D frame for each potential compound. The SIEVE software found 593 frames for the trend analysis of the APCI-files.

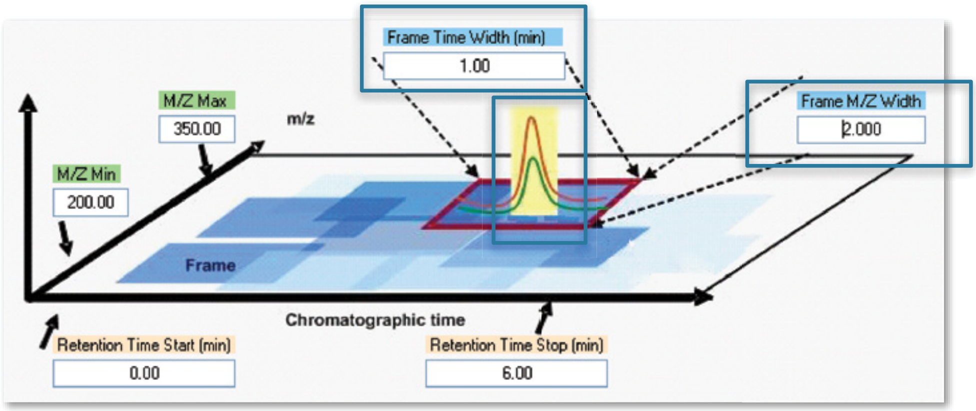

Frame Time Width (min): 1.00
M/Z Max: 350.00
M/Z Min: 200.00
Frame M/Z Width: 2.000
Retention Time Start (min): 0.00
Retention Time Stop (min): 6.00

Figure 1. Example of a SIEVE frame

By analyzing this series of frames along with a control compare trend experiment to eliminate background signals from the sample, TP candidates can be identified. The control compare trend experiment compares a control of several samples in which a variable has been modified, allowing monitoring of the E1 signal and its intermediates as a function of the variable known to affect the formation of TPs. After applying data filters, the list of candidates can be reduced to a manageable number of peaks.

Further analysis based on a set of three rules for 3D frame selection narrowed this group to 16 frames and then to two frames based on their peak intensity and acceptable peak shape. The rules help to reject compounds that were present in a sample prior to ozonation, ensuring that selected compounds are not randomly appearing in samples but are observed consistently, and to enable targeting of more abundant compounds that have sufficient intensity for LC-HRAM.

Structural elucidation of two identified compounds

Elemental composition using a Qual Browser program of the Thermo Scientific™ Xcalibur™ 2.1 software was determined for the two selected compounds, TP-276 and TP-318, and compared to E1. Structural elucidation by LC-HRMS was then performed to discover the differential structures of the compounds and how they were formed from the ozonation reaction.

Figure 2. Elemental composition of the two frames of interest

Frame #	*m/z*	Elemental composition	RDBE*	Δmmu
E1	269.15537	$C_{18}H_{21}O_2$	8.5	0.667
#9	275.12930	$C_{16}H_{19}O_4$	7.5	0.418
#78	317.14008	$C_{18}H_{21}O_5$	8.5	0.633
		$C_{11}H_{25}O_{10}$	-0.5	-5.240

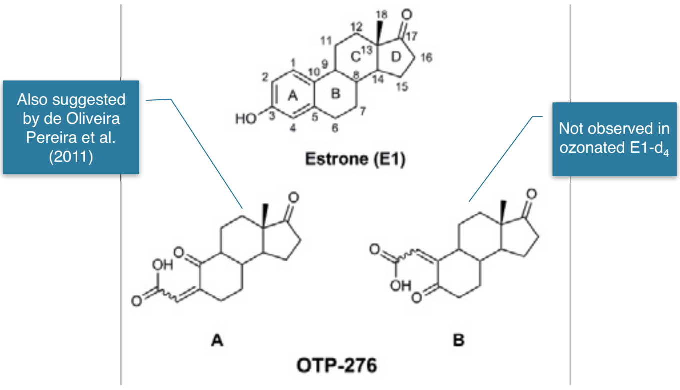
Loss of 2 C and 2 H atoms and additional of 2 O

Most likely structure: addition of 3 O

*RDBE: Ring and double bond equivalents

Δmmu: experimental error in millimass units

Multi-stage tandem mass spectrometry (MS^n) experiments of the ozonated E1 and deuterium-labeled analogue (E1-d4) samples were performed. They generated the same TPs with different masses in order to confirm the sites on the E1 molecule where the reaction with O3 occurred and propose structures of the MS^n product ions of the identified E1 TPs. MSn showed that TP-276 and TP-318 had carboxylic acid and hydroxyl functional groups, similar to that previously reported for TPs of other endocrine disruptors. Structures for these two compounds were proposed based on their MS^n spectra.



Also suggested by de Oliveira Pereira et al. (2011)

Not observed in ozonated E1-d_4

Figure 3. Structural elucidation of TP-276 by LC-HRAM

Though the two elucidated compounds appear to be correct transformation products for estrone, they fail to explain the issue of the initial spike in estrone activity for low ozone dosages. Both structures displayed open phenolic rings, of which the closed formation signifies the active functional group for estrone activity, indicating these products should not have estrogenic activity. Supplemental analysis on TP formation early in the reaction will be required.

Conclusion

The new LC-HRAM approach has been proven to allow efficient identification of TPs, opposing traditional thinking that monitoring the removal of parent compounds is sufficient and that TPs are not persistent. The method simplifies current procedures and enhances reproducibility with the addition of a control compare trend experiment followed by differential analysis and MSn. The structural elucidation and subsequent analysis of TP formation from the original ozonation reaction supports previous data regarding reaction mechanisms and how contaminants of emerging concern can generate transformation products.

For more information, watch the video, "LC-MS as a Tool for Engineers Optimizing Wastewater Treatment", presented by Dr. Viviane Yargeau – Department of Chemical Engineering & McGill School of Environment, McGill University, Montreal

Resources in addition to Webinar Presentation: https://www.epa.gov/wqc/contaminants-emerging-concern-including-pharmaceuticals-and-personal-care-products

References

SEGURA, P.A., KAPLAN, P., YARGEAU, V. (2013) Identification and structural elucidation of ozonation transformation products of estrone, Chemistry Central Journal, 7:74.

ADDITIONAL RESOURCES

Webinar

Multi-Residue Targeted Method for the Analysis of CECs in Wastewater Using the Q Exactive HR/AM-Mass Spectrometer

Poster

Screening and Quantitation of Micro-pollutants from Sewage Water in the Process of Bank Filtration Using UHPLC-HRAM

Articles

Identification of Transformation Products of Organic Contaminants in Natural Waters by Computer-Aided Prediction and High-Resolution Mass Spectrometry

High-Resolution Mass Spectrometry Method for the Detection, Characterization, and Quantitation of Pharmaceuticals in Water

Rapid Quantification of Pharmaceuticals and Pesticides in Passive Samplers Using Ultra High Performance Liquid Chromatography Coupled to High Resolution Mass Spectrometry

Brochures

Thermo Scientific SIEVE Software for Differential Expression Analysis

Thermo Scientific LTQ Orbitrap XL Hybrid FT Mass Spectrometer Unrivaled Performance and Flexibility

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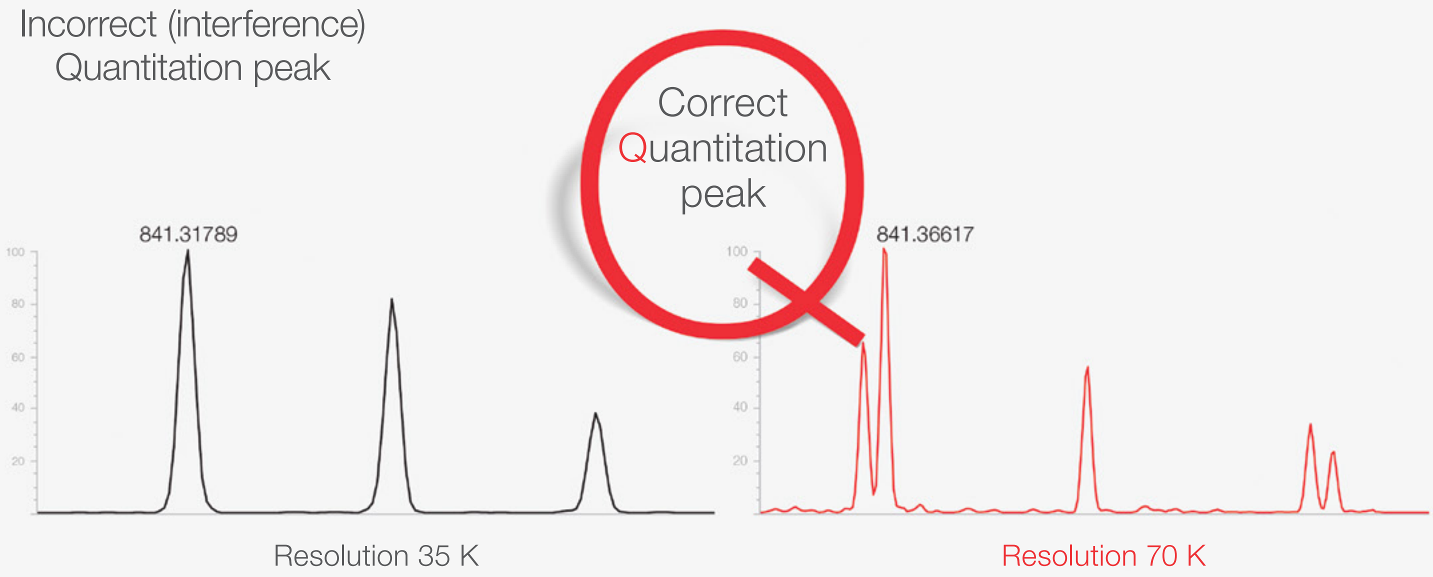

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Mass Spec Reveals Decades-old Hidden Pollutants

Careful sampling and high-res mass spectrometry unearthed an ecological nightmare in the waters of Nueces Bay in Texas.

Authors: Hussain Abdulla and Dorina Murgulet (Texas A&M University) Richard F. Jack and Dwain Cardona, Thermo Fisher Scientific, Inc.

The Texas oil boom of the early 20th century transformed the fortunes of the state. Yet the gusher age has also left a lasting and damaging impact on the region's ecology.

Until 1993, the discharge of petroleum brine – a byproduct of oil extraction containing hydrocarbons and dissolved minerals – occurred routinely along the Texan coast. In 1979, for example, it was estimated that over 32 million gallons of petroleum brine were released daily into Texan tidal waters.

One area where the legacy of petroleum brine discharge is still being heavily felt is Nueces Bay – a shallow microtidal bay in Southwestern Texas. Despite a ban on the release of petroleum brine into U.S. surface waters in 1993, the area remains severely ecologically affected, with species such as oysters still unable to grow over two decades after the last of the effluent was released.

The inability of the bay to fully recover from the effects of historical petroleum brine discharge has puzzled environmentalists for years. Studies fail to elucidate the source of toxicity in the bay, thus minimizing the success of any restoration efforts and proper evaluation of strategies to enhance habitat health. However, new research conducted by a Texas A&M University-Corpus Christi team may potentially shed light on this ecological mystery. Their findings, obtained using high resolution accurate mass spectrometry, suggest an underlying ecological problem that has been in the making for decades.

There's something in the water

To gain insight into the causes of Nueces Bay's ecological problems, the Texas A&M-Corpus Christi team decided to probe the chemistry of the water. Here, the team needed an approach that would quickly and accurately identify all of the organic components present in the samples collected.

The group collected water samples at 15 sites along two transects situated in the north and south of the bay (Figure 1). The organic compounds present in the water samples were removed by solid phase extraction.

The team leveraged the analytical speed and mass accuracy of the latest Thermo Scientific™ Orbitrap™ technology to comprehensively identify the organic components present in the water samples. They also needed to ensure that their approach captured all of the chemical components in the water samples. Using an untargeted, full scan acquisition approach, the team were able to detect between 1800 and 4000 unique compounds depending on sampling location.

One of the most striking findings was the disparity in the distribution of compounds between the two transects. A greater number of unique molecular formulas were detected at sampling points in the northern transect relative to the southern transect. While the number of unique carbon-, hydrogen-, and oxygen-containing molecules was similar for both regions, the number of unique phosphorus-containing compounds was significantly higher in the water samples collected at sampling sites along the northern transect (Figure 2). In some cases, over five times as many organophosphorus compounds were detected.

To further understand these findings, the team used van Krevelen plots to determine the chemical nature and origin of the molecules detected. These diagrams are two-dimensional graphical representations that can reveal the chemical identity of the compounds based on the ratio of oxygen atoms to carbon atoms (O/C) and ratio of hydrogen atoms to carbon atoms (H/C).

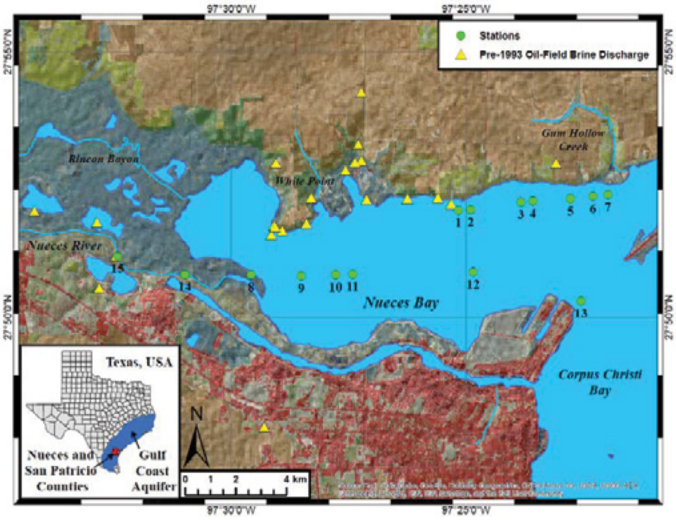

Figure 1. Location of sampling sites in Nueces Bay

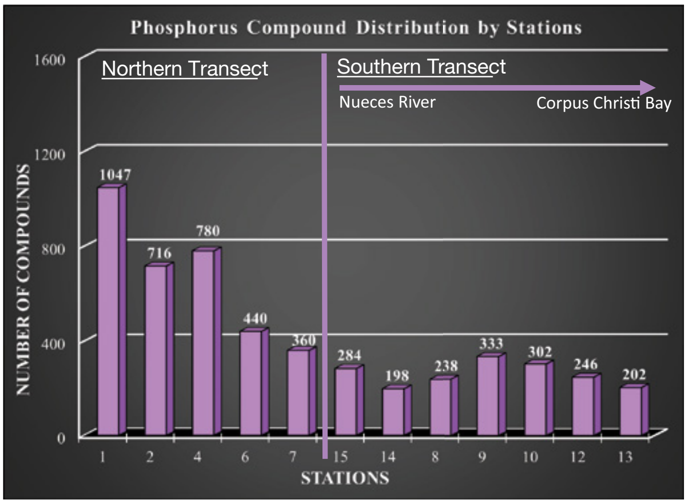

Figure 2. Number of unique phosphorus-containing compounds at sampling sites 1 to 15

As well as a high abundance of oxygenated hydrocarbons at sampling stations located close to former discharge sites, consistent with the bay's history of extensive petroleum brine release, the plots revealed a more surprising result. A large proportion of phosphorus-containing compounds with low H/C ratios were also detected, indicative of aromatic organophosphorus molecules. These compounds tended to fall outside the range for naturally occurring phospholipids and phosphopeptides in the van Krevelen diagram, suggestive of manmade origin.

A toxic combination

Abdulla's team believe that the high abundance of organophosphorus compounds at sampling sites located along the northern transect originates from the agricultural land-use in areas to the north of the bay (see the brown colored open land in Figure 1). Many pesticides and agricultural products have historically been rich in phosphorus-containing compounds. Due to surface and/or subsurface agricultural runoff, these compounds have subsequently found their way into the bay.

Yet many of the phosphorus-rich pesticides identified by the team have now been banned, so what could explain their reappearance? The answer may lie in the bay's history of petroleum brine discharge.

In addition to hydrocarbons, petroleum brine is rich in iron – an element with a high affinity for organophosphorus and phosphates. The team believe that a large proportion of the pesticide molecules present in the agricultural runoff would have become adsorbed on the bay sediment particles that have become enriched in iron during petroleum brine discharge. Over time, and with sedimentation, these insoluble organophosphorus containing iron deposits would have become buried beneath layers of sediment and hidden away for decades.

The reason for the recent reappearance? It could be due to changes in microbial activity. Bacteria present in the sediment are known to use a number of metabolic pathways to oxidize the organic matter they use to live and reproduce. While oxygen is their preferred oxidizing agent, once supplies run low they typically move onto other oxidants such as ferric iron (Fe^{3+}), which they reduce to ferrous iron (Fe^{2+}). In the oxygen starved sediment at the bottom of Nueces Bay, the team believe that the previously insoluble ferric iron complexes are being converted to the more soluble ferrous form – resulting in the release of the adsorbed organophosphorus molecules into the water.

Trapped by the iron-containing sediment and buried for decades, it appears that these now banned pesticides and their degradation products are slowly being released into the waters of Nueces Bay. The team's findings suggest that the ecological problems currently observed in the bay are a result of ecological damage decades in the making, which are only now beginning to surface, thanks to the latest advances in full scan high resolution accurate mass instrumentation.

Looking to the future

To more fully understand the extent of the brine contamination problem, additional work is underway over the wider Nueces Bay area.

"Using liquid chromatography coupled with high resolution accurate mass spectrometry, we plan to comprehensively identify most of the organophosphorus compounds present in the bay's waters," explains Abdulla.

The team hopes to determine location-specific concentrations to obtain a more complete picture of the situation. Abdulla and his team also want to understand how resilient the pollutants are to degradation.

"Given the large number of unique phosphorus-containing molecular formulae—between 800 and 1000 in some locations—it's likely that many of the identified compounds are degradation products rather than original pesticides," Abdulla says.

As these compounds are exposed to sunlight upon being released into the water from the iron-containing sediment, investigations into the compounds' photooxidation degradation pathways are underway.

Thanks to the latest advances in high resolution accurate mass spectrometry, Abdulla's team have cast light on an ecological problem that has been buried beneath Nueces Bay for many decades. It's hoped the team's full scan approach may be used to comprehensively profile environmental pollutants present in other surface waters.

Published in Laboratory Equipment, November 2017

ADDITIONAL RESOURCES

Poster note
A Strategy for an Unknown Screening Approach on Environmental Samples Using Mass Spectrometry

Brochure
Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

Learning center
Emerging Contaminants Analysis

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