Environmental Compendium

Volume 3

SCIEX

The Power of Precision

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SCIEX Environmental Compendium Volume 3

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Introduction

SCIEX Environmental Compendium Volume 3

Environmental analysis effectively forms a line of defense that protects the wider public by detecting toxic or harmful environmental contaminants before they cause adverse health problems. Such contaminants include per- and polyfluoroalkyl substances (PFAS) and their derivatives, as well as residual pesticides or pharmaceuticals in soil, drinking water or wastewater samples.

Environmental analysis involves detecting substances that are detrimental to health in various samples, even if the chemicals are present at ultra-low concentrations. It is imperative for environmental scientists to have methodology that can help uncover unknown contaminants or quantify known substances that could pose significant risk to the unsuspecting public.

Characterization lies at the heart of identifying and quantifying potential contaminants in samples. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been widely adopted across the industry due to its ability to deliver reliable, reproducible results, even for trace contaminants. From the routine analysis of soil for pesticides to sampling air and water for persistent organic pollutants, mass spectrometry offers robust identification and quantification of these molecules.

SCIEX provides a comprehensive portfolio of mass spectrometry instruments to fit the needs of industry regulators, and academic and contract laboratories. This Environmental Compendium demonstrates the utility of SCIEX instruments and expertise across various applications by highlighting specific methods useful in the environmental analysis industry, such as identifying regulatory pesticides, PFAS, residual pharmaceuticals, microcystins and other toxic aquatic substances.

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Alex Liu

Market Manager, Food and Environmental Testing

Identifying the most prevalent, persistent and harmful contaminants is an essential component of environmental analysis. Quantifying these dangerous chemicals relies on a combination of accurate screening instrumentation and understanding existing regulation that identifies the safe levels permitted in our environment.

Environmental analysis now typically involves monitoring samples from multiple locations to identify anything that could potentially impact, or have been impacted by, humanity. It is common to perform analytical tests on samples of drinking/waste water, air, and soil in order to identify potential contaminants, such as pesticides and particulate matter, as well as detect new emerging contaminants.

Emerging contaminants present significant challenges to scientists, as this category of chemicals corresponds to those compounds or chemicals suspected of presenting a risk to the public. One of the most high-profile examples is the series of compounds known as per- or poly-fluoroalkyl substances (PFAS). These man-made flame-retardant chemicals have been specifically designed to be non-degradable, and have been identified in samples of drinking water and other environmental samples across the globe. Even polar bears, who live in some of the most remote regions of the world, have been exposed to PFAS. There is also growing evidence that exposure to PFAS can lead to adverse health effects in humans. This has made regulating their presence in the environment all the more important, and in 2018 the United States Environmental Protection Agency (EPA) imposed limits of 70 parts per trillion on perfluorinated compounds. For any scientist needing to identify these compounds, and any other emerging contaminant, it is important to adapt quickly to the changing regulatory landscape.

"There is a growing focus to detect not only the contaminant chemicals, but also to identify the secondary molecules such as the metabolites"

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As well as emerging contaminants, there are also those contaminants that are routinely sampled. For instance, pesticides are essential to provide sufficient food from harvests. However, residual presence in soil or drinking water can have adverse environmental and health effects. For this reason, it is common for laboratories to routinely test for chemical contaminants such as pesticides or waste pharmaceutical products in water supplies.

Broadly speaking, the analysis of environmental samples is handled in one of three settings: research laboratories, industrial contract laboratories and regulatory bodies. A contaminant will typically be first identified during routine research and will join the emerging contaminant list. It is then the job for research laboratories to investigate the risk the chemical poses to the surrounding environment and wider population. Typically, they will employ the most sensitive and modern instrumentation to identify and quantify the new chemicals and compounds. If the risk posed is significant, this research will then influence local or national government regulation. Industrial or contract laboratories must then fulfill this regulation by testing environmental samples to ensure restricted compounds are below imposed levels.

Within environmental analysis laboratories, liquid chromatography is increasingly being used over its counterpart, gas chromatography, to separate individual analytes for analysis. There is a growing focus to detect not only the contaminant chemicals, but also to identify the secondary molecules such as the metabolites. The growing field of omics research has led to what is coined as exposomics, which involves studying every potential contaminant in the environment that humans may be exposed to. As these topics grow in popularity, the impetus will be on using novel and innovative technologies. Even in the past few years, there has been a shift in the analytical methodology used, with more emphasis placed on non-targeted screening to identify multiple potential contaminants within a single analytical run. The next generation of mass spec instrumentation will be one that prioritizes sensitivity and robust characterization.

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A Global Perspective

In all regions of the world, environmental analysis is needed. Whether it is to test drinking water samples for PPCP's and pesticides to ensure conformance with state regulations, it could be to test soil for residual contaminants or whether it is looking at the presence of persistent organic pollutants in the raw waters such as lakes and rivers. Here we present a global perspective from our experts in environmental testing looking at what in their region.

Ashley Sage
Senior Market Development Manager, EMEAI

Yuka Ikoma
Market Development Manager, Japan

Kerong Zhang
Senior Market Development Manager, China

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Chris Hodgkins
Market Development Manager & Sales, Australia

Global Trends in Environmental Testing

Paul Winkler, Market Development Manager, Americas

Per- and poly-fluoroalkyl substances (PFAS) are a major concern amongst US regulatory bodies and researchers in the environmental field.. Now that a few high-profile PFAS have been regulated, industries that require these powerful surfactants are instead turning to homologues, GenX for example. Little is known about the toxicity of the latest generation of PFAS, and identifying and quantifying this range of thousands of compounds presents a challenge to environmental testing laboratories. Algal toxins are another area of increasing interest in North America, particularly in coastal regions and the Great Lakes area. Some of these toxins are found on the Unregulated Contaminant Monitoring Rule (UCMR 4) , the latest watch-list from the Environmental Protection Agency (EPA), and regulations will be drafted if any of these compounds are identified in significant quantities.

Triple quadrupole instruments continue to be very effective at targeted screens of known environmental contaminants. However, in order to analyze environmental samples for the large range of PFAS or algal toxins that may be present but are not on a target compound list, non-targeted screening is required using high-resolution mass spectrometry on a QTOF X500R instrument. The X500R is simple to use, fits on a benchtop and offers a wide linear dynamic range that provides excellent quantification; features that perfectly fit the needs of environmental laboratories. Over the next few years I believe we will see a paradigm shift away from targeted screens towards analyzing environmental samples for entire libraries of compounds, and the QTOF X500R can deliver this need.

Ashley Sage, Senior Market Development Manager, EMEAI

Around Europe, the primary application for SCIEX LC-MS/MS instruments is in water quality testing. There is a large number of pollutants that can find their way into drinking water. Industrial effluent may contain persistent organic pollutants, pesticides used on crops will leech into ground water, and a large quantity of pharmaceuticals in human waste are flushed into sewers every day. Municipal and federal agencies, as well as industries, are greatly invested in protecting the consumer through ensuring water quality. For their part, regulatory bodies are looking to increase the number of compounds that are monitored in water sources and set limits for acceptable levels of these compounds.

SCIEX provides a variety of instruments for the different needs of environmental testing laboratories. Much routine environmental analysis can be achieved with a QTRAP 4500 in MRM acquisition mode. For trickier analytes such as estrogens, the QTRAP 6500+ provides the required sensitivity. European water testing laboratories are also at the forefront of non-targeted screening, in which entire libraries of over 500 compounds are analyzed in a single sample. The QTOF X500R can identify all these compounds in a simple workflow. As environmental regulations become increasingly stringent, we are going to see testing of more compounds at lower concentrations, and we provide the tools our customers need to achieve this.

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Jason Neo, Director, Marketing & Field Applications Support, Rest Of Asia (ROA)

Governments all around the Asia Pacific region are increasingly focused on analyzing water samples for contaminants in order to protect the health of the general public. Government laboratories routinely screen samples for a range of compounds classified as pharmaceutical and personal care products (PPCPs). Similarly to the United States, there is also increasing concern around PFAS contaminants in water. Regional Environmental Protection Agencies are implementing more stringent PFAS testing and most countries around Asia are now monitoring PFAS.

The QTRAP systems provide best-in-class sensitivity and workflow for routine analysis of environmental contaminants such as PPCPs and PFAS. QTRAP systems offer excellent sensitivity and selectivity without the need for extensive sample preparation. In particular, the QTRAP 5500 with an MS/MS library is the gold standard for environmental testing in ROA. We believe in strongly supporting our customers, and the SCIEX ROA field application team works very closely with water testing labs on developing new methodology and workflows to improve their analysis.

Kerong Zhang, Senior Market Development Manager, China

In China, with its rapidly developing industry, there is increasing public concern around pollutants in water sources, including pesticides, hormones and pharmaceutical and personal care products (PPCP). Antibiotics are of particular concern, and have been detected in major rivers around the country. In recent years, the Chinese government has implemented a series of environmental water quality standards, and researchers require the right tools to monitor and control these serious pollutants.

SCIEX offers expertise not just in instrumentation and analysis software, but also in our development of robust methodologies. Methods developed by SCIEX researchers can aid our customers in the detection and quantification of a wide swath of environmental pollutants. Our scientists have developed methods for detection of all pollutants listed in Chinese water quality standards using LC-MS/MS triple quadrupole systems. We also offer methodologies for detection of more than 200 pesticides, a library of PPCPs, and nitrosamines, common byproducts of water disinfection. These methods can greatly improve the workflow of our customers, and save them valuable time and money.

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Yuka Ikoma, Market Development Manager, Japan

As a society, we use innumerable chemicals every day. This use is not confined to industry, but also in consumer goods such as shampoos, food packaging, cosmetics and clothes. Many of these chemicals end up in the environment as pollutants and it can take a long time before their environmental impact becomes evident and fully understood. Given these conditions, environmental scientists in Japan are seeking tools that give them broad coverage of chemical contaminants. This will enable them to uncover the full list of compounds present in environmental samples and build a fuller picture of the human exposome; every chemical humans are exposed to.

We have seen adoption of SCIEX QTOF instrumentation using SWATH technology in Japan, as a solution to the challenges outlined above. By employing this technology, researchers are able to detect and quantify more chemicals than ever before in a single analysis. This enables environmental testing laboratories to obtain a better understanding of the human exposome, giving researchers a better chance to deal with potential pollutants.

Chris Hodgkins, Market Development Manager & Sales, Australia

Australia has a unique ecosystem, and it's important that we do all we can to protect it for future generations. Thankfully, Australia has not experienced any major disruptive environmental contamination recently, and that reflects the high-quality work performed in environmental testing labs. In particular, Australia has very high standards for drinking water, and local authorities routinely perform extensive testing to maintain delivery of a safe water supply.

Like other countries around the world, there is ongoing concern about the level of PFAS found in the environment. A focus of testing labs here is on facilitating remediation work on sites where PFAS-containing fire-fighting foams were previously used. One of the challenges with this analysis is that many different PFAS have been produced and used, meaning non-targeted screening is required to fully understand the extent of environmental contamination. We believe we are going to see a big increase in the use of the QTOF X500R in coming years, for analyzing PFAS and other emerging contaminants.

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Technical

Overview

Exposomics is a rapidly growing field examining the health effects of every environmental contaminant that humans are exposed to from birth until death, to fully understand the threat of a lifetime of exposure to innumerable compounds.

Only through strict monitoring, using the most sensitive and robust instrumentation, can environmental scientists discern exactly what is in our soil, water and air. From routine monitoring of algal toxins in water supplies, to complex analysis of the output from oil extraction, the work of environmental testing labs is both challenging and diverse.

In order to meet the needs of the environmental testing community, SCIEX has designed and developed some of the most advanced mass spectrometers in the industry. Through in-house research in a variety of environmental contexts, SCIEX has repeatedly shown that it can deliver reliable and accurate results with the speed and simplicity required by modern testing labs.

Here we present an overview of the latest SCIEX technologies, many of which are specifically designed to address challenges in the expanding field of environmental science.

The X500R QTOF Series

Scientists carrying out environmental testing require techniques that can offer high-throughput and sensitive detection capabilities in order to analyze environmental samples quickly. To meet these needs, SCIEX developed the revolutionary X500R Series of QTOF mass spectrometers. Its key features include enhanced mass accuracy to deliver improved selectivity for routine mass spectrometry (MS) quantitation of targeted compounds, combined with sensitive and high-speed MS/MS data acquisition to enhance quality of data and ensure comprehensive detection of unknown compounds. This accuracy is due to the X500R series' Turbo V™ ion source, which has a renowned ionization performance among industry professionals.

In addition, the SCIEX X500R Series has an independent calibrant delivery path, which maintains highly reliable mass calibration through long runs. Together, these features provide the platform with the speed and mass accuracy required for the high throughput identification and quantitation of environmental analytes. These advantages make the X500R system well suited for non-targeted screening, an area of growing importance to environmental testing labs. For detail on accomplishments using the X500R Series, please see the relevant Technology Notes in this compendium.

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The QTRAP® 4500 Series

Intelligently re-engineered from the 4000 QTRAP® platform, the SCIEX QTRAP 4500 LC-MS/MS system offers environmental scientists vigorous and reliable high-throughput screening for a wide range of analytes. Designed for robustness, rapidity and quantitative sensitivity, the QTRAP 4500 is well suited for situations where trace analyte detection is required, a common need for identifying environmental contaminants such as pesticides. Similarly to the X500R Series, it possesses a Turbo V ionization source - the gold standard for LC-MS/MS ionization. Plus, with the 4500, investigators can maximize the capacity of their laboratories and deliver high quality quantification every time. This is due to the QTRAP 4500's powerful Scheduled MRM™ algorithm and Curved LINAC® collision cell design, which, together, improve the quality of data to ensure fewer peaks are missed and optimal sensitivity is achieved.

The QTRAP 6500+ System

For the most difficult, challenging environmental applications, such as the analysis of poor ionizers including polar pesticides, you need the most sensitive instrument available. The QTRAP 6500+ System is the fastest and most sensitive QTRAP system available, delivering enhanced selectivity and improved levels of quantitation. It features the ability to eliminate background interferences to make quantitative analyses easier, as well as IonDrive™ technology to boost performance in those situations where exceptional selectivity is a necessity. This single instrument pushes the boundaries of LC-MS/MS further than ever before and provides a simple route to achieving comprehensive environmental sample monitoring.

The CESI 8000 Plus

With origins dating back to the 1990s, capillary electrophoresis (CE) has been used in multiple scientific domains and has proved to be vital in the analysis of environmental samples for residual pesticides. The SCIEX CESI 8000 Plus, in particular, has been used as a platform for transformative chiral separation techniques. The CESI 8000 Plus provides a unique multi- segment injection system, which offers a ten-fold increase in the throughput of an analysis.

CE is well suited to the separation of polar samples and Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS) offers a methodology that can improve retention times of samples for better detection. As will be detailed later in this compendium, the technique has been used to develop quantitation and identification methodology for polar pesticides, commonly considered to be amongst the most difficult to quantify.

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KC Hyland

Staff Scientist, Food and Environmental Testing

KC Hyland is a Global Staff Scientist for SCIEX Food and Environmental, placing her at the forefront of innovation. Here, she discusses the various applications of mass spectrometry in the field of environmental testing and how it will evolve in the future.

One of the biggest challenges facing the environmental analysis industry is undoubtedly detecting per- and polyfluoroalkyl substances (PFAS). These chemicals are ubiquitous, with items such as stain resistant carpets, food packaging and waterproof jackets among the types of household items that contain PFAS. The chemical stability of the molecules means that PFAS are very persistent in the environment, and, once present in oceans or waterways, can be transported over long distances. This makes detecting these compounds a global issue.

PFAS are a well-known series of chemicals. Dating back to the 1940s, they have been used for decades in various industrial applications due to their resistance to water, fire and oil. Present day interest in these compounds is driven by wide public concern and new regulations that have placed limits on the presence of PFAS in samples. Two of the most well-known examples are perfluorooctane sulfanoate (PFOS) and perfluorooctanoic acid (PFOA), which were outlawed in the late 2000s. However, as these two molecules are phased out, structurally similar replacement molecules are being introduced to serve a similar function. Environmental analysts are now focused on detecting and quantifying these new chemicals, which are generally referred to as GenX compounds.

Alongside PFAS are other contaminants that are more routinely analyzed by environmental scientists. For instance, pesticides will continue to be used so long as mankind needs food.

"Using a QTOF X500R for instance, scientists are able to identify nearly every contaminant in their sample, by screening against growing libraries of known PFAS or GenX compounds"

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However, as the chemicals themselves change, so too do sample preparations and detection methodologies. Examples of further anthropogenic contaminants that require analysis include chemicals introduced during the process of fracking, or oil sands process-affected water produced during surface mining.

Natural toxins can also pose a potential threat. Among the most common are algae in oceans or freshwater that can naturally produce microcystins or domoic acid. These toxins can contaminate seafood before progressing up the food chain. Microcystin contamination in particular is not a new phenomenon. Detection of these contaminants has always been necessary owing to the adverse health effects associated with these toxins. However, natural events are driving interest in detection methodologies. Algae blooms have reportedly been on the rise as a consequence of warmer waters caused by climate change. This has resulted in the detection of algae toxins being of increased concern for many environmental laboratories across the world.

The industry is also adapting detection and analysis methodologies to match the advances in instrumentation. The standard detection method used in laboratories involves analyzing known analytes in a sample, for instance using Triple Quad mass spectrometers. This method has been highly successful over the years in analyzing those contaminants known to scientist, such as PFOS and PFOA chemicals or pesticides. Now modern instrumentation and analytical methods are taking this analysis one step further. Non-targeted analysis is becoming increasingly popular in industrial and academic laboratories across Europe and China. Using a QTOF X500R for instance, scientists are able to identify nearly every contaminant in their sample, by screening against growing libraries of known PFAS or GenX compounds. Non-targeted analysis represents the future of environmental mass spectrometry for identification and quantifying analyses.

Environmental analysis relies on detecting both the known environmental threats such as pesticides, while also adapting instrumentation and detection strategies to future emerging risks. With new technological and industrial processes continuously being developed, it will only become more essential for regulators, academics and contract laboratories to work together to identify and monitor contaminants in the air, water and ground.

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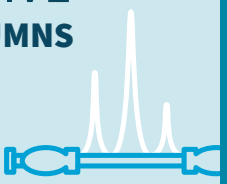
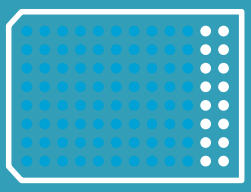


250X LESS SOLVENT

LESS SAMPLE NEEDED



Less sample is needed per run so you can get more from your samples

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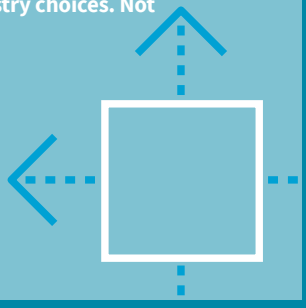

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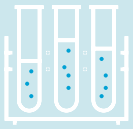

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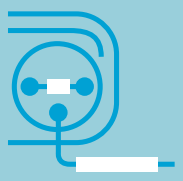

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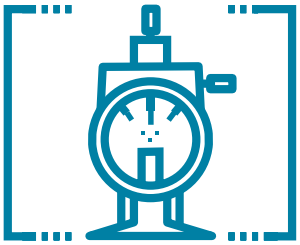

An Overview of SCIEX Technology Notes

A Robust and Sensitive Method for the Direct Analysis of Pesticides in Food and Environmental Samples Without Derivatization

Multi-residue LC-MS/MS analyses have become the minimum requirement for the quantification of pesticides in food and environmental samples. Modern tandem quadrupoles enable labs to quickly analyze large quantities of environmental samples for many regulated pesticides.

However, very polar pesticides, such as glyphosate, present significant challenges for these current methods. They are frequently poor ionizers, suffer from low extraction from the sample matrix and demonstrate poor chromatographic separation. Therefore, polar pesticides typically require complex single-residue methods that involve considerable derivatization and clean-up steps.

NofaLab, an independent Dutch sampling laboratory, has developed a robust high-throughput LC-MS/MS assay for detection of many of these polar pesticides and their metabolites in food samples, without derivatization. In collaboration with SCIEX, this method has been extended using a SCIEX QTRAP® 6500+ for direct injection of water samples. The new method is sensitive enough to detect low pesticide quantities to meet the stringent regulatory limits for environmental samples.

Analysis of PFAS in drinking water with EPA Method 537.1 and the SCIEX QTRAP® 4500 System

Exposure to high levels of per- and polyfluoroalkyl substances (PFAS) has been associated with reproductive complications, immune responses and some forms of cancer. The Environmental Protection Agency (EPA) method 537.1 outlines the sample preparation, reporting guidelines and quality control of various PFAS in drinking water.

Although EPA 537.1 has prescriptive guidelines for PFAS sample preparation, the guidelines for the LC-MS/MS analysis allow more flexibility. Working within these guidelines, SCIEX has developed a method for PFAS analysis using the QTRAP® 4500 system. Column chemistry, chromatography, mobile phases and MS/MS transitions were all optimized to improve PFAS analysis workflow and performance. The method delivers short runtimes as well as robust, reproducible results. It is also highly sensitive, meeting or exceeding the requirements of the EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) list for drinking water for the entire suite of substances defined by EPA 537.1.

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LC-MS/MS Solution for Determination of Nitrosamine Disinfection Byproducts in Drinking Water

Nitrosamines are carcinogenic organic compounds widely used in the manufacture of cosmetics, pesticides and rubber. One nitrosamine, NDMA, a known water disinfection byproduct, has been categorized as a Group 2A carcinogen by the International Agency for Research on Cancer.
The World Health Organization (WHO) has established a limit of 100 ng/L NDMA in drinking water. Public concern around this compound
remains high, and a simple solution for monitoring drinking water is required.

Using the SCIEX QTRAP® 4500 LC-MS/MS system, a method has been developed for quantifying eight nitrosamines in a simple and rapid fashion, allowing monitoring of NDMA levels across the water supply chain. The method has high sensitivity, with a detection limit 10-fold lower than that required by the WHO. It can also be performed on any SCIEX QTRAP® or Triple Quad™ systems, cutting method development time. With simple sample pretreatment and short runtimes, this method provides accurate and quantitative analysis of nitrosamine disinfection byproducts in drinking water.

Rapid Characterization of Naphthenic Acids Using High Resolution Accurate Mass MS and MS/MS with SelexION™ Differential Mobility Separation

Extraction methods from oil sands produce large quantities of oil sands process-affected water (OSPW) as a byproduct. OSPW consists of a complex matrix of organic contaminants, including

naphthenic acid fraction components (NAFCs), which are currently of great concern to environmental scientists. As regulation in this area becomes implemented, it is important that testing laboratories can analyze these samples efficiently.

OSPW extracts have high complexity and as a result, traditional chromatography approaches for their separation require long runtimes and multiple handling steps. Direct infusion of OSPW into ultrahigh resolution mass spectrometers can give useful NAFC profiles, however further steps are required for structural elucidation of analytes. To meet these challenges, SCIEX has developed a unique workflow for NAFC analysis using a TripleTOF® 5600+ high resolution accurate mass system coupled with SelexION® differential mobility spectrometry.

SelexION separates hard to resolve ions based on their gas phase mobility. Compared with other chromatographic alternatives, it provides a cheaper, greener method for NAFC separation from OSPW extracts. Coupled to the TripleTOF 5600+, rapid and accurate information on structural isomers can be obtained.

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Quantitation and Identification of Legal and Illicit Drugs in Wastewater in the low Nanogram per Liter Range using Large Volume Direct Injection and QTRAP® Technology

Drug abuse is a global problem affecting millions worldwide. Drugs of abuse not only pertain to those drugs manufactured and bought illegally, but also addictive prescription drugs such as morphine and benzodiazepines. Analyzing wastewater for drugs of abuse has become an important tool for monitoring drug consumption trends.

SCIEX has optimized existing LC-MS/MS methodology for analyzing wastewater for the presence of both legal and illegal drugs, as well as their metabolites. Using a SCIEX QTRAP® 5500 system in multiple reaction monitoring (MRM) mode, drug compounds were measured with a detection limit in the ng/L range, a 10-fold improvement over non-optimized methods. The method was successfully applied to measure drug levels in influent wastewater collected during a street festival in Zurich.

Quantitation of Dicamba and Acid Herbicides in Agricultural Field Samples on SCIEX QTRAP® 6500+ System

Acid herbicides (AcHs), such as dicamba, are widely used to control weeds on residential lawns and in industrial agriculture. While regulations around the use of AcHs are currently limited, they remain of concern to the scientists who require robust methods to quantify these herbicides in environmental samples.

Current methods for analyzing AcHs involve complex derivatization steps with separation and detection by gas chromatography and electron capture. This process is both time consuming and difficult to perform correctly. SCIEX aspired to overcome these challenges through development of a new LC-MS/MS method for AcH analysis.

Using the highly sensitive SCIEX QTRAP 6500+ in ESI- mode, quantitation of AcH compounds was achieved to ng/L concentrations. This method does not require analyte derivatization and therefore reduces both time and complexity.

High Resolution Quantitation of Microcystins and Nodularin on SCIEX X500R QTOF System

Microcystins and nodularins are carcinogenic classes of compounds produced by cyanobacteria and aquatic microfaunal communities. As a matter of public health, it is important to monitor both environmental samples and drinking water for their presence.

Environmental testing labs frequently employ triple quadrupole systems for this analysis, but often need more confirmatory information than these systems can provide in order to identify contaminants. To this end, SCIEX has developed an LC-MS/MS method using the high resolution and accurate mass capabilities of the X500R QTOF system, which maintains the capacity that environmental testing labs require to carry out routine analysis. This new method was used for accurate quantitation of a suite of eight microcystins and one nodularin in water samples at concentrations well below the EPA's Minimum Reporting Levels (MRLs).

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Improving Identification and Quantification of Polar Herbicides by CESI-MS

Glyphosate is a common herbicide associated with various health risks and environmental damage. More stringent regulations have recently been introduced by the European Union to restrict its presence in the environment and reduce harm to human health and ecosystems. New methods are required for more accurate detection in environmental matrices.

Traditional LC-MS analysis methods frequently require time-consuming derivatization steps to detect glyphosate and other polar pesticides, and can also have difficulty distinguishing between different degradation products. Researchers at SCIEX endeavored to develop a new, more effective method for this separation and detection.

The new Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS) method provides excellent specificity for distinguishing glyphosate and its degradation products. It can also readily distinguish the degradants of glyphosate and another common herbicide, fosetyl aluminum.

TRAINING AND SERVICES FOR SCIEX

Realize Your Lab's Potential

It can be difficult to manage the everyday challenges of an analytical lab, contending with an increasing volume of food samples for analysis while ensuring any potential contaminant is detected. SCIEX is here to help. Our Lab Optimization Services can help you increase your throughput, reduce your costs and improve quality by identifying and removing unproductive activity.

Building on over 15 years of experience working in food testing labs, we can help identify areas to eliminate unproductivity across various workings including method development and refinement, routine troubleshooting, data or sample processing, and other areas. We have a proven track record, with our clients reporting productivity increases as high as 50% and a corresponding saving of capital.

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In addition to maintaining laboratory equipment, it's vital to maintain your employees' knowledge. In a testing environment where every analyte must be identified and quantified using a variety of different mass spectrometers, your technicians must have the knowhow to accurately assess results and deliver concise summaries.

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Tech Notes

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A Robust and Sensitive Method for the Direct Analysis of Polar Pesticides in Food and Environmental Samples Without Derivatization

Wim Broer[1], Ugo Chiuminato[2], Jianru Stahl-Zeng[2], Daniel McMillan[3] and Phil Taylor[3]
[1] Nofalab Laboratories, Schiedam, NL; [2] SCIEX, Darmstadt, DE; [3] SCIEX, Warrington, UK

The Challenge of Polar Pesticides

The prevalence of multi-residue LC-MS/MS analyses for the quantification of pesticides in food and environmental samples has been steadily increasing for many years, and they are now considered to be a minimum requirement of most laboratories working in these fields. Modern tandem quadrupoles are capable of detecting such regulated compounds at very low levels with minimal sample preparation, such as QuEChERS, thereby enabling labs to process large numbers of samples for many analytes with a fast turnaround. However, some very polar compounds which are not amenable to the extraction procedure, chromatographic method or are poor ionizers require additional single-residue methods which involve time-consuming preparation and separation and often involve derivatization to improve detection.

Key Advantages Presented

- All analytes were well retained, allowing detection of the majority of background components which could otherwise interfere. Separation between the analytes was also sufficient to allow unambiguous identification, and retention times were reproducible. Sensitivity in spiked environmental waters was found to be similar to that in standards, and the target limit of detection of 20 ng/L was easily achieved with real drinking water samples.
- Matrix effects were largely eliminated in both the NofaLab method for food sample extracts and the modified method for direct injection of water samples. Use of QTRAP® is expected to confirm positive results by their full-scan MS/MS spectra, but future work will investigate different or additional clean-up.

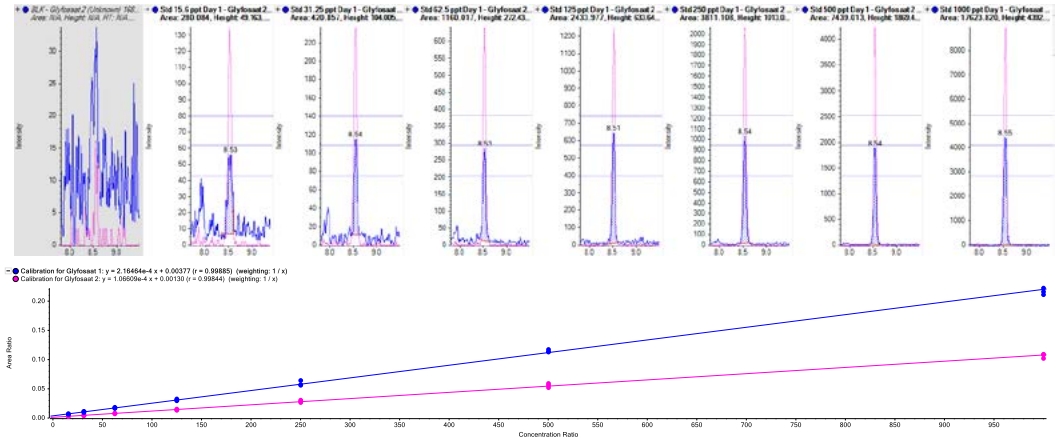

Figure 1. **Method sensitivity and linearity of glyphosate.** Calibration standards in concentrations from 15.6 to 1000 ng/L of glyphosate achieved using the modified method for water samples. Ion ratios were all well within the specified ±20% tolerance.

Contents

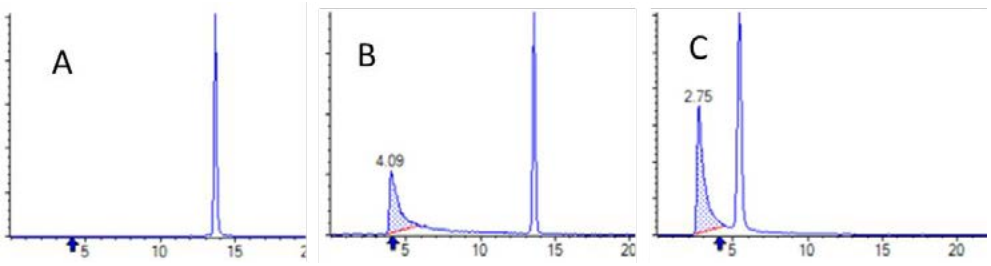

Figure 2. Use of a preferred column means: Install, Prime, Repeat, and finally Replace. Image A shows the performance of the preferred column after installation, no glyphosate peak is present. Image B shows the same column after it has been conditioned with 30 spinach extracts, a glyphosate peak can be detected at 4.09 mins. Image C shows retention time (RT) drift of the glyphosate peak on the same column after 100 injections.

Growing Concerns

Recent increase in public concern regarding the presence of glyphosate has significantly increased the requirement to analyse it and its metabolites in food, feed and the environment, so has accelerated the need for a more efficient and robust analytical method. The extraction and chromatography of these compounds is well described in the EURL-QUPPE method, but the separation is not robust in practice, so system and method maintenance are intensive. Several different HPLC or HILIC based methods have failed to address the issues of reproducibility and sensitivity, so FMOC derivatization prior to analysis is often still employed for glyphosate, AMPA and glufosinate. Although possible to automate, this procedure is still time consuming or expensive, and is not applicable to the other polar pesticides of interest.

Creating a High Throughput Method

NofaLab is an independent sampling and testing laboratory based near Rotterdam, Netherlands, specializing in the fields of food, feed and environmental safety. The increasing pressure to provide fast, quantitative analysis has driven NofaLab to add to their portfolio of LC-MS/MS instrumentation and develop a new method which covers as many of these polar pesticides in a single analysis as possible. Ion chromatography has been shown to be beneficial for separation, but the need for a suppressor is detrimental to MS analysis and the inefficiencies of changing inlet systems on a heavily used mass spectrometer makes it impractical in a busy lab performing primarily reverse-phase LC.

So, the final method, presented here, makes use of an LC column in a method-switching reverse phase (RP) system with MS amenable mobile phases at around pH 9. Such conditions configure glyphosate ideally for MS detection with good retention and separation of the other analytes and matrix interferences. The method meets the DG-SANTE[1] requirements of reproducibility (<20%) and recovery (80-110%), and the LOD of the method is below 0.01 mg/kg. Excellent long-term stability and robustness were achieved throughout the validation of this method for food samples extracted by the QUPPE procedure.

Where environmental samples require testing, the regulatory limits are much lower5 and interference from matrix more problematic in traditional analyses with a short retention time, so derivatization is often the only option. However, since glyphosate is well retained in this new method, the potential to further develop it for direct large-volume injection was investigated in collaboration with SCIEX. By modifying the gradient conditions and optimizing the injection parameters, a second method specific to environmental water samples has been developed. Although the large volume injection (LVI) is more susceptible to changes in pH (for example, due to evaporation of mobile phase) robustness has been shown to be similarly good, and allows detection of the same suite of analytes with a LOD of <0.02 ng/l.

Experimental Considerations

Food samples

The QuPPe method for extraction of polar pesticides from samples of plant and animal origin developed by Anastassiades et al. at CVUA Stuttgart[2] are well described and have undergone several revisions. Since the analytes are water soluble, it is based on aqueous extraction with addition of methanol and formic acid to improve efficiency.

The addition of internal standards is essential to compensate for the shifting retention times in most chromatographic method and helps to counter matrix effects where present. This was particularly important for grain and seed samples, where

chromatographic performance deteriorates, and the MS source becomes dirty, losing sensitivity quickly, so dispersive C18 cleanup as described in the QuPPe-AO3 method was attempted before finalizing on a push-through method with two sorbents using SPE filters.

Various chromatographic methods have been investigated and found to have several limitations. Figure 2 illustrates the common practice of extensive conditioning prior to analysis, which after relatively few (typically 30-50) sample injections in order to maintain peak shape and retention time Ion chromatographic methods showed most promise, but the eluents' incompatibility with electrospray ionization sources requires the use of a suppressor, which is detrimental to peak width. However, by employing a polyvinyl alcohol based column with quaternary ammonium groups and using an ammonium bicarbonate buffer prior to detection by a very sensitive quadrupole mass spectrometer, the need for a suppressor is removed.

Table 1. List of food matrices used for method verification.

Lists of Validated Commodities	
A	*Fruit and Vegetables*
B	*Seeds*
C	*Vegetable oil, Fat and Fatty Acids*
D	*Grain*
E	*Herbs and spices*
F	*Meat and Seafood*
G	*Animal Oil, Fat and Fatty Acids*
H	*Eggs and Eggs products*
I	*Milk and Milk products*
V	*Fatty acids*

Method verification was performed on a variety of food matrices (Table 1), all subject to clean-up as described above. Performance was robust and reproducible with 10µl injections, but peak shape started to deteriorate after around 200 samples, with significant distortion appearing by the 350th injection due to the limited capacity of the 2mm i.d. column. The final chromatographic method uses a 150 x 4mm column and employs a guard column of the same material and a 0.5µm filter, both of which are replaced every 250 samples to maintain performance and to keep the MS source clean.

Water samples

Environmental and drinking water samples varied widely in the degree of comprised particulate matter, which causes difficulties for LC injection and is detrimental to reproducibility. However, minimal sample preparation is desirable in a high throughput laboratory situation and SPE type clean-up would add significant time and financial cost. In order to overcome these challenges, a simple filtration step using Chromacol 17-SF-02 (RC) from 17 mm syringe filters was performed when transferring samples to the LC vials. Internal standards to a final concentration of 1ppb were added to samples and standards, and QC samples in tap water were prepared in a similar fashion. Experiments were also performed using standard addition to the samples to investigate any potential matrix effects.

Separation was achieved using a Shimadzu Nexera UHPLC system comprising LC-30AD pumps, a SIL-30AC autosampler fitted with a 500µL loop and a CTO-20A column oven. An injection volume of 500µL was employed in a chromatographic method similar to that used for the food samples. During verification of the method, the primary focus was on achieving stable peak shapes and retention times for all analytes. Loop size (irrespective of injection volume), initial conditions, gradient and pH of the mobile phase had very significant effects, so the final optimized method should be fixed, and fresh mobile phases prepared regularly.

Method verification was performed with real drinking water samples, testing for both AMPA and Glyphosate, a LOQ of 20ng/L could be reached.

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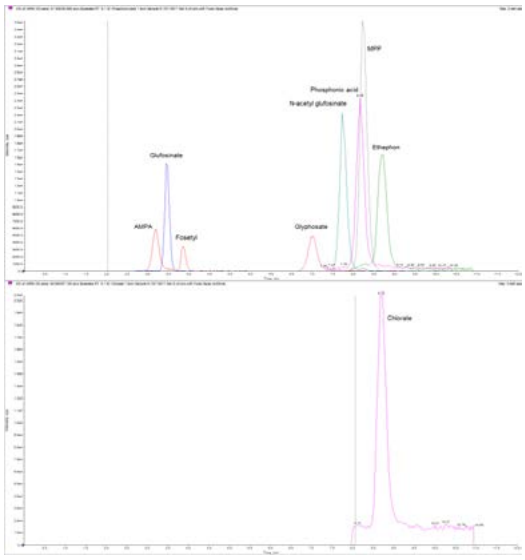

Figure 3. Example chromatograms shown for polar pesticides suite. Chromatographic separation using the hypercarb column was an integral component of the described method.

Table 2. Source parameters for the SCIEX QTRAP®® 6500+ System.

Source Parameters	
Curtain Gas (CUR)	*30 psi*
Collison Gas (CAD	*9 psi*
IonSpray Voltage (IS)	*-3000v*
Temperature (TEM)	*500°C*
Ion Source Gas (GS1)	*55 psi*
Ion Source Gas (GS2)	*65 psi*

MS-MS Analysis

Analyses were performed using a SCIEX QTRAP®® 6500+ mass spectrometer in negative electrospray ionization mode. At least two MRM transitions were optimized for each analyte as outlined in Table 3 in order to quantify and confirm their concentration in all samples. Data was acquired using Analyst® 1.6.3 .and processed for quantitation and confirmation with reference to internal standards using MultiQuant™ 3.0.2 software.

Table 3. List of analytes with MRM transitions employed. Internal standards are crucial to this method and must be used.

Analyte	Q1 m/z	Q3 m/z
Glyphosate 1	167.9	150.0
Glyphosate 2	167.9	78.8
Glyphosate 3	167.9	62.8
Ethephon 1	142.9	106.8
Ethephon 2	142.9	79.0
N-ac Glufosinate 1	222.0	136.0
N-ac Glufosinate 2	222.0	62.8
N-ac Glufosinate 3	222.0	59.1
AMPA 1	110.0	81.2
AMPA 2	110.0	79.1
AMPA 3	110.0	62.9
Glufosinate 1	180.0	136.0
Glufosinate 2	180.0	95.0
Glufosinate 3	180.0	85.0
Glufosinate 4	180.0	63.1
3-MPPA 1	151.0	132.9
3-MPPA 2	151.0	107.0
3-MPPA 3	151.0	63.1
Phosphonic Acid 1	81.0	62.9
Phosphonic Acid 2	81.0	79.0

Contents

Results and Discussion

Food samples

Chromatographic performance using both the NofaLab method for QuPPe extracts of food samples and the modified method for water samples achieved good separation between the analytes and from matrix interferences, and excellent repeatability in terms of peak profile and retention time. The EU maximum residue limits for these compounds in food samples range from 10 to 2000 µg/kg, depending on the commodity and compound[4], so the target for each is variable. Although water regulations are under discussion, a detection limit of 20 ng/L for environmental samples is desirable in anticipation of future regulation. Some analyte/matrix combinations proved to be particularly difficult, but these target concentrations were easily achieved for all samples in the verification of the methods. Over 1000 food samples from a variety of commodities were analyzed at NofaLab without maintenance of the system, and the stability in terms of retention time, peak width, peak area and tailing factor was found to be excellent. Figure 1 shows several measures of reproducibility based on the glyphosate internal standard.

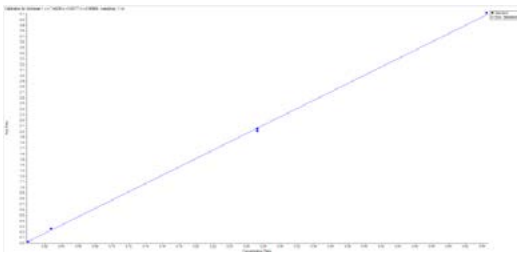

Figure 4. Glyphosate calibration standards. Linear calibration regression for glyphosate with 1/x weighting, showing r-value of 0.9997 and excellent precision for duplicate calibrators.

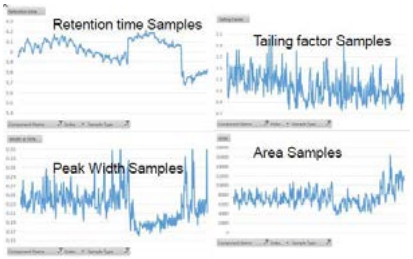

Figure 5. Reproducibility data for glyphosate IS. NofaLab method for food samples, tested over 1000 injections of extracts from fruit/veg, seeds, veg oil/fat, grains, herbs/spices, meat/fish, animal oil/fat, eggs/egg products, milk/milk products and other fatty acids.

Table 4. Summary of Limits of Detection achieved in various food matrices using the NofaLab method. Shown along with their EU Maximum Residue Limits[1].

Product	Glufosinate sum			Fosetyl sum			Glyphosate			Chlorate			Ethephon		
	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL
Fruit and Vegetables	16	30	11%	25	2000	13%	5	100	15%	8	10	15%	18	50	11%
Seeds	12	30	12%	90	2000	15%	8	100	15%	3	10	10%	6	50	14%
Vegetable oil, Fat and Fatty Acids	15	30	19%	40	2000	12%	7	100	22%	2	10	6%	3	50	7%
Grain	18	30	12%	71	2000	14%	8	100	7%	7	10	14%	9	50	6%
Herbs and spices	25	100	8%	87	2000	13%	23	100	6%	8	10	15%	8	100	16%
Meat and Seafood	19	30	15%	23	100	12%	9	50	23%	4	10	8%	4	50	10%
Animal Oil, Fat and Fatty Acids	14	30	20%	51	100	11%	9	50	25%	10	10	16%	7	50	12%
Eggs and Eggs products	18	30	12%	33	100	11%	4	50	13%	12	10	9%	6	50	17%
Milk and Milk products	17	30	9%	20	100	6%	8	50	22%	5	10	12%	5	50	13%
Fatty acids	21	100	14%	70	1000	14%	3	100	18%	4	10	9%	3	100	10%

Water samples

To achieve the target sensitivity for environmental water samples, it was necessary to inject increase the amount of sample, so trials with increasing injection volume and different loop sizes were carried out. With each incremental change, the composition of eluent in the loop was altered, thereby changing initial conditions of the analysis and the retention times and peak shapes of the analytes. To compensate, modification of the stating composition of the mobile phase was required, but when final parameters had been fully developed, the method was found to be as stable and robust as the NofaLab method for food samples. All analytes were well retained, allowing detection after the majority of background components which could otherwise interfere had eluted. Separation between the analytes was also sufficient to allow unambiguous identification, and retention times were reproducible. Sensitivity in spiked environmental waters was found to be similar to that in standards, and the target limit of detection of 20 ng/L was easily achieved with real drinking water samples. In order to verify the results, analyses with standard addition of the target compounds were also performed.

Matrix effects were largely eliminated in both the NofaLab method for food sample extracts and the modified method for direct injection of water samples. However, MRM ion ratios were found to be outside of the normal ±20% tolerance in some very complex sample matrices. Use of the QTRAP® will be advantageous to confirm positive results by their full-scan MS/MS spectra, but future work will investigate different or additional clean-up of samples in order to remove background interferences.

Conclusions

This ion chromatographic approach to the analysis of polar pesticides offers the ability to include multiple analytes in a single injection without derivatization. Deviating from traditional LC buffers has enabled detection by MS/MS and the sensitivity of the SCIEX 6500+ QTRAP® mass spectrometer allowed the analysis to be performed without the need for an ion suppressor using a standard reverse-phase LC based system. Therefore, the need to change inlets between typical pesticide analyses is eliminated, allowing high-throughput laboratories to manage samples efficiently and minimize running costs. System maintenance was found to be within expectations, with a change of guard column only required after approximately 250 sample injections.

The methods were found to be considerably more robust and sensitive than other approaches described in various publications and have achieved the target limits of detection required to meet existing and proposed future regulations. The separation has been found to minimize matrix interferences in most samples, but further work will investigate possible improvements to clean-up in order to achieve confirmatory results in even very complex matrices.

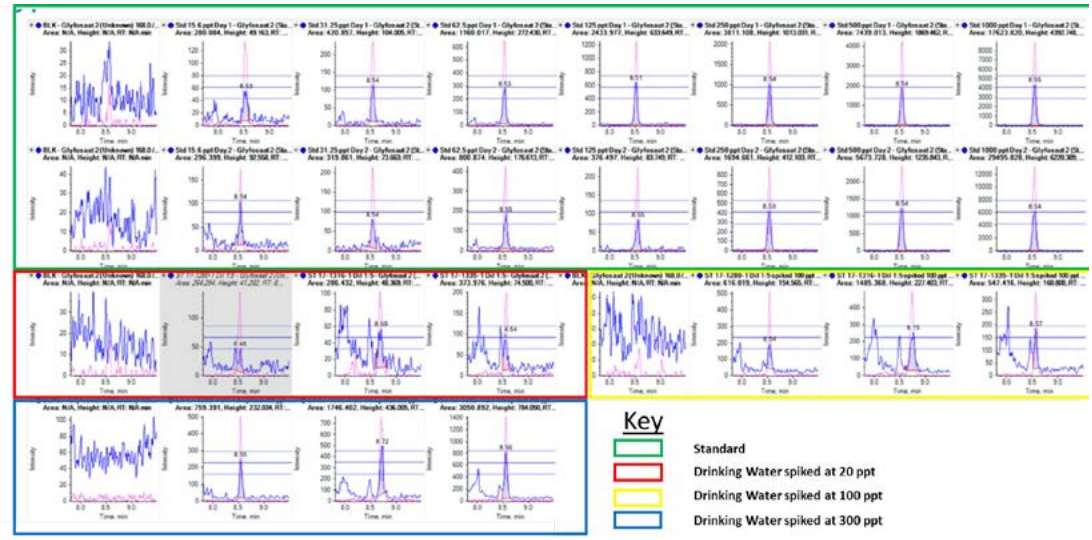

Figure 6. Example chromatography from drinking water samples using the modified water method.

References

1. https://ec.europa.eu/food/plant/pesticides/glyphosate_en
2. http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf
3. http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN
4. http://ec.europa.eu/environment/archives/ppps/pdf/ma_reding_annex4.pdf
5. Glyphosate and AMPA in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality

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Contents

Quantitation of Dicamba and Acid Herbicides in Agricultural Field Samples on SCIEX QTRAP® 6500+ System

Herbicides and their Metabolites in Soy and Soil

KC Hyland, Paul Winkler
SCIEX, Redwood City, CA

Widespread global use as weed control agents and plant growth regulators for agricultural crops, lawns, and gardens makes the active ingredients in Acid Herbicide (AcH) products account for more use than all the other types of pesticides combined. These predominant herbicide chemicals include the well- characterized 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, triclopyr, and other AcHs. The US EPA recently ruled in favor of continued use of dicamba despite complaints and concern about drift across plots during spray application. While adjustments in regulation around application patterns were made, these ACHs remain a prevalent concern in environmental monitoring and crop contamination analysis.

Historically, the analysis of AcHs has been performed using a complex sample preparation procedure to derivatize the analytes followed by detection with gas chromatography and an electron capture detector. The US EPA Method 8151: CHLORINATED HERBICIDES BY GC USING METHYLATION OR PENTAFLUOROBENZYLATION DERIVATIZATION has been the most common analytical approach for these analytes. This method, however, is extremely difficult to perform correctly, is not

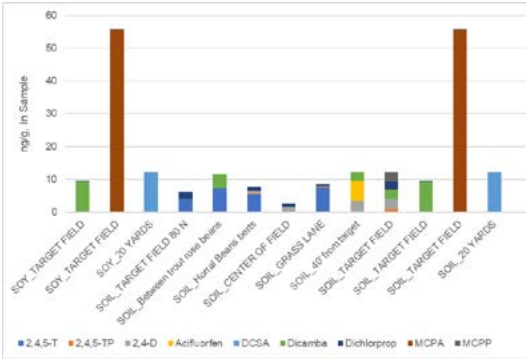

Figure 1. Quantitation of acid herbicides and metabolites in real-world samples. Measured concentrations of some detected AcHs in the field-collected soy foliage and soil samples. LOQs for the described method range from 0.1 – 140 ng/g in matrix. Samples were collected not only from targeted fields, but also from increasing distances from the field center.

rugged and is time consuming. LC-MS/MS as a replacement technology would eliminate the need for the derivatization step thus making this a more rugged analytical approach. A recent literature review of chlorophenoxy acid herbicide methods demonstrated that LC-MS/MS was the prevalent technology cited. Acidic functional groups are easiest to ionize as their conjugate base, and LC-MS/MS methods can utilize negative mode electrospray ionization (ESI−) with great sensitivity.

Key Points

- Quantitation was achieved to ng/L concentrations for many analytes in neat calibration solutions, corresponding to ng/g levels in field samples.
- An isotopic internal standard, d3-Dicamba, was employed to assess recovery, precision, and robustness of the method. The ISTD peak area %CV was 21% across both soy foliage and soil matrices.
- Recoveries were generally between 70-150% and replicate precision were within %CV of 20%.
- Important dicamba metabolites 5OH-Dicamba, DCSA, and DCGA were included in the analytical method.

Contents

Experimental

Sample Preparation:

5 g of soil sample or soybean foliage were collected from impacted and non-impacted agricultural field sites. Internal standard was added to the sample pre-extraction. Sample was homogenized and extracted with formic acid fortified acetonitrile. Sample was shaken for 15 minutes then centrifuged at 4000 rpm. The supernatant was diluted with aqueous mobile phase into 2 mL amber autosampler vials for LC-MS/MS analysis.

HPLC Conditions:

Chromatographic separation of these highly polar, low molecular species was achieved using a Phenomenex Kinetex F5 column. Excellent analyte retention and peak quality is demonstrated using this relatively novel stationary phase, and a 17-minute gradient program (Table 2) provided chromatographic resolution for performance in complex extract matrices.

MS Conditions:

A SCIEX QTRAP® 6500+ system was employed for its sensitivity and robustness. Optimized MRM transitions were selected and utilized for maximum sensitivity. Isotopically labelled target analytes were utilized as internal standards for achieving the highest quality quantitation data in complex soil and plant extracts. Table 1 details the instrument conditions utilized in this method.

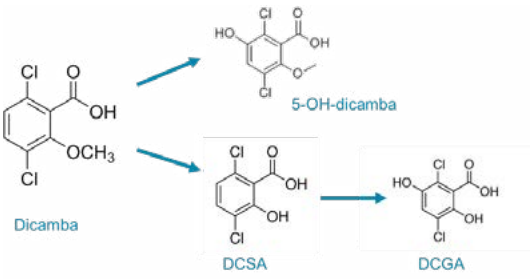

Figure 2. Major dicamba metabolites are also relevant. Metabolites of concern are 5-OH Dicamba and 3,6-dichlorosalicylic acid (DCSA). DCSA is the major degradate in the environment and is more persistent in the environment than the parent Dicamba. DCSA can further transform into DCGA.

Table 1. Ion Source Parameters. Electrospray Ionization (ESI) conducted in positive ion mode.

Parameter	Setting
Curtain Gas (CUR)	*20*
Collision Gas	*10*
Ion Spray voltage (IS)	*5500*
Temperature (TEM)	*650*
Nebulizer Gas (GS1)	*50*
Heater Gas (GS2)	*50*

Results

Chromatography:

The F5 stationary phase demonstrated excellent retention and resolution for these small, polar species. The LC gradient (Table 2) was utilized to maximize separation from matrix interferences. RT values were specified for each MRM transition to optimize cycle time for best peak shape and quantitation. Figure 3 shows example elution profiles.

Table 2. LC Gradient time program.

Time (min)	% B
1	*40*
4	*52*
12	*85*
13.5	*90*
15.5	*90*
15.6	*2*

Quantitation:

Acid herbicide LODs (Limits of Detection) were found to be mostly <1 ng/mL, with some exceptions including 5OH-dicamba, which had an LOD of 1 ng/mL. Isotopically labelled d3-Dicamba was utilized as an internal standard for all analytes. The calibration range was 0.025 - 50 µg/L (Figure 4).

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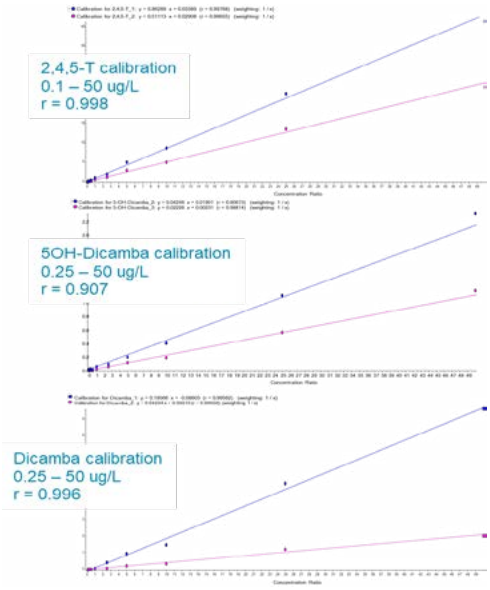

Figure 4. Calibration curves of some example AcHs demonstrating sensitivity, linear response, and dynamic range.

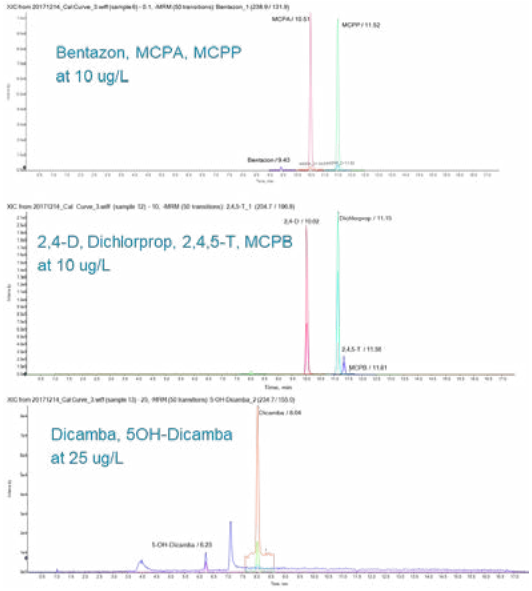

Figure 3. Elution profile of some example AcHs using the Kinetex F5 stationary phase.

Analytical precision, determined using triplicate injections at varying concentration levels, is shown in Table 3 and Figure 5. The reproducibility of the isotopic ISTD was 21% CV in matrix samples. This value includes peaks measured in both soy foliage and soil matrices representing excellent method reproducibility in matrix.

Table 3. Quantitative method performance for acid herbicides and metabolites, including sensitivity and reproducibility data.

Compound ID	LOD (ng/mL, in vial)	LOQ (ng/mL, in vial)	LOQ (ng/g, in sample)	S/N at 1ppb	%CV at 1ppb	%CV at 25ppb	Cal Range
2,4,-T	0.1	0.25	3.5	132	12%	11%	0.1 - 50
2,4,5-TP	0.025	0.05	0.7	72	18%	6%	0.025 - 50
2,4,-D	0.025	0.05	0.7	226	6%	7%	0.05 - 50
2,4-DB	5	10	140	--	--	3%	5 - 50
5OH-Dicamba	1	2.5	35	49	26%	3%	0.5 - 50
Acifluorfen	<0.1	0.1	1.4	17	10%	11%	0.1 - 50
Bentazon	<0.01	<0.01	<0.14	1883	5%	3%	0.1 - 25
DCGA	5	10	140	--	--	7%	--
DCSA	1	2.5	1.4	7	7%	8%	0.05 - 50
Dicamba	0.25	1	14	25	14%	11%	0.25 - 50
Dichlorprop	0.025	0.05	0.7	586	2%	5%	0.025 - 50
MCPA	1	2.5	<0.14	4	1%	3%	0.01 - 100
MCPB	0.5	1	14	384	6%	2%	0.5 - 50
MCPP	<0.01	<0.01	<0.14	560	3%	3%	0.01 - 100

Contents

Data Collected from Field Samples:

Occurrence data were collected from various spatial targets within impacted and non-impacted fields in the US Midwest. Occurrence data were collected for soy plant tissue (“foliage”) and soil.

- 7 soy foliage samples
  - Target field planted rows, and increasing distances from field
- 5 soil samples
  - 3 from target field, and 2 from increasing distances

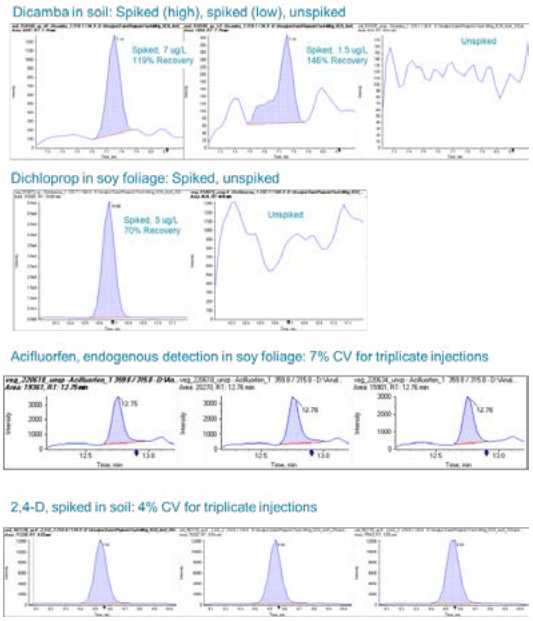

Figure 5. Spike recovery for dicamba and other acid herbicides in soil and soy matrices, as well as analytical precision for triplicate injections.

2,4,5-T and Dichloprop were detected most frequently; 2,4-D was detected in most vegetation samples but not in any soil samples. MCPA showed high concentration detected in one of each type of sample. Samples were collected not only from targeted fields, but also from increasing distances from the field center. One finding of note was the presence of the metabolite DCSA in samples 20 yards from the center, but not at the center (Figure 1).

Summary

The SCIEX QTRAP® 6500+ system was coupled with the ExionLC™ AD and Phenomenex Kinetex F5 analytical column to attain sensitive quantitation of acid herbicides including dicamba and dicamba metabolites. Quantitation was achieved to ng/L levels for many analytes in neat calibration solutions, corresponding to ng/g levels in the field samples. Spiked and unspiked agricultural samples were analyzed to demonstrate sensitivity, recovery, and precision in complex matrices. Target field samples demonstrated highest frequency of analyte detection compared to samples collected further from fields. Endogenous occurrence of several analytes was reported.

References

1 EPA Method 8151A. 1996.

2 Guo et al., Talanta, 2015.

3 Sack et al., J. Ag. Food Chem. 2015.

Acknowledgments

The authors and SCIEX thank and acknowledge and Ping Wan for providing field collected samples and Leah Riter for expertise on the target analyte list.

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Quantitation and Identification of Legal and Illicit Drugs in Wastewater in the low Nanogram per Liter Range using Large Volume Direct Injection and QTRAP® Technology

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Overview

The present application note describes the optimization of the front-end HPLC methodology by improving the separation of legal and illicit drugs such as cocaine, MDMA and methamphetamine and its important metabolites such as benzoylecgonine and monoacetylmorphine using an unusual 5 µm particle core-shell column with 4.6 mm ID. The SCIEX QTRAP® 5500 system was used to detect target compounds in Multiple Reaction Monitoring (MRM) mode. To overcome saturation effects observed for high sensitive MRM transitions the collision energies (CE) were detuned for some compounds. Different acquisition modes such as *Scheduled* MRM™ and *Scheduled* MRM™ Pro with Information Dependent Acquisition (IDA) of MS/MS full scan spectra were explored. Thus, the analytes could be quantified in a traditional way using two MRM transitions in MultiQuant™ 3.0 software, and additionally, QTRAP® MS/MS spectra could be used for identification at trace levels in MasterView™ 1.1 software. The optimized method was successfully applied to the measurement of drugs in influent wastewater samples collected during a party event (Street Parade Zürich).

Introduction

Drug abuse is a global problem with major negative impacts on human health and social welfare. Illicit drugs are substances for which nonmedical use is prohibited by national or international laws. Important groups of illicit drugs are opioids, cocaine, cannabis, amphetamines and ecstasy (MDMA). Among those, amphetamines and MDMA currently demand the most attention by law enforcement agencies.[1] In Europe, the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) is the reference point on drugs and drug addiction.[2]

For drug consumption, questionnaire-based surveys have traditionally been performed to estimate drug use. However, it is recognized that this method is not sufficient to monitor trends in drug use quickly and adequately and therefore complementary data from other sources are needed.[3]

Since several years, the chemical analysis of influent-wastewater for the combined excretions products of illicit drugs has become a potent approach for monitoring patterns and trends of drugs consumed in a community.[3, 4] Meanwhile, the study of spatial differences and temporal changes in illicit drug use through the method of wastewater analysis, also called sewage epidemiology, is becoming an important tool to estimate drug consumption in Europe.

Nowadays, LC-MS/MS has become the method of choice for the quantitative determination of illicit drugs in aqueous matrices.[6] The *Scheduled* MRM™ algorithm using unique fragment ions and specific retention times of the molecules has evolved as a promising method for the reliable quantitation of compounds in water matrices.[7] Large volume direct injection (LVDI) techniques together with the exceptional sensitivity of the SCIEX QTRAP® 5500 system allow limits of quantitation (LOQ) in the low ng/L range.[8, 9] Finally, the acquisition of MS/MS spectra using Enhanced Product Ion scanning (EPI) in the Linear Ion Trap of the QTRAP® mass spectrometer provides additional confidence of the presence of the analytes under investigation.

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The Street Parade Zürich is an interesting event to study such trends because the wastewater influent is strongly affected due to the relationship between number of inhabitants (approx. 300000) and visitors (approx. 950000).

Experimental

Standards and Internal Standards (IS)

Target analytes (morphine, monoacetylmorphine, amphetamine, methamphetamine, codeine, monoacetylcodeine, MDMA, cocaine, benzoylecgonine, methadone, EDDP, and mephedrone) as well as their corresponding deuterated IS were obtained as solutions in methanol or acetonitrile from Lipomed, Arlesheim, Switzerland. Working standard and calibration solutions were freshly prepared by appropriate dilution with methanol and water (purified using a water purification system from ELGA, Villmergen, Switzerland).

Sampling and sample preparation

Wastewater samples were obtained from the Zürich-Werdhölzli sewage treatment plant (STP) and immediately acidified to pH 2 using HCl, filtered and stored in the dark at 4°C until analysis. For the analysis 1 mL of wastewater sample was transferred to an HPLC vial, diluted 1:1 and 1:10 respectively, with ELGA water, and 10 µL of deuterated IS added. The final concentration of the IS was 500 ng/L. For ion suppression studies samples were also diluted 1:1 or 1:10.

Samples were collected as 24 h composite influent wastewater samples over 7 days between Wednesday July 30th (SP1) and Tuesday August 5th, 2014 (SP7). Sample SP 4 corresponds to the day of the Street Parade event and SP 5 to the day after.

LC Separation

A Dionex Ultimate 3000 HPLC system with a binary gradient pump, autosampler and column oven (30°C) was used for the chromatographic separation.

The LC method was completely redesigned. In the previous method a Phenomenex Synergi Hydro-RP column 100 x 2.1 mm 2.5 µm was used.[6] In this study, a core-shell LC column, Phenomenex Kinetex C18, 100 x 4.6 mm, 5 µm, was applied. Mobile phase A was water + 0.1% formic acid + 2 mM ammonium formate and mobile phase B acetonitrile. A flow rate 900 µL/min was used. The gradient with a total run time of 12 minutes is listed in Table 1. The injection volume was set to 100 µL.

Table 1. LC gradient

Step	Time (min)	A (%)	B (%)
0	0.0	98	2
1	1.0	98	2
2	7.0	35	65
3	7.1	0	100
4	9.0	0	100
5	9.1	98	2
6	12.0	98	2

MS/MS Detection

A SCIEX QTRAP® 5500 system with Turbo V™ source with ESI probe was used. The target compounds were detected in positive polarity. The ion source parameters were optimized for the new LC conditions using the Compound Optimization (FIA) function in Analyst® software.

Table 2. Ion source parameters

Parameter	Value
Curtain Gas (CUR)	30 psi
IonSpray voltage (IS)	3000 V
Temperature (TEM)	650°C
Nebulizer Gas (GS1)	70 psi
Heater Gas (GS2)	70 psi

Two characteristic MRM transitions were monitored for each analyte, and 1 MRM transition for each internal standard (Table 3). The MRM transitions were taken over from the existing method[6] and MRM transitions of mephedrone and the corresponding IS were added.

The *Scheduled* MRM™ algorithm was activated to monitor compounds only around the expected retention time to maximize dwell times and optimize the cycle time of the methods.

Table 3. MRM transitions and retention time (RT)

Compound	RT (min)	Q1	Q3
Morphine (MOR)	3.4	286	152
	3.4	286	165
Codeine (COD)	4.2	300	215
	4.2	300	165
Amphetamine (AMP)	4.3	136	91
	4.3	136	119
Monoacetylmorphine (MAM)	4.5	328	165
	4.5	328	211
Methamphetamine (MAMP)	4.5	150	91
	4.5	150	119
MDMA	4.6	194	163
	4.6	194	105
Mephedrone (MEP)	4.7	178	160
	4.7	178	145
Benzoylecgonine (BEC)	4.9	290	168
	4.9	290	105
Monoacetylcodeine (MAC)	5.2	342	225
	5.2	342	165
Cocaine (COC)	5.4	304	182
	5.4	304	105
EDDP	6.4	278	234
	6.4	278	249
Methadone (MET)	6.7	310	265
	6.7	310	105
IS Morphine	3.4	289	152
IS Codeine	4.2	303	215
IS Amphetamine	4.3	139	122
IS Monoacetylmorphine	4.5	331	165
IS Methamphetamine	4.5	155	92
IS MDMA	4.6	199	165
IS Mephedrone	4.7	181	163
IS Benzoylecgonine	4.9	293	171
IS Monoacetylcodeine	5.2	345	225
IS Cocaine	5.4	307	185
IS EDDP	6.4	281	234
IS Methadone	6.7	313	268

The *Scheduled* MRM™ pro algorithm in Analyst® software (version 1.6.2) was used as an alternative survey scan for information dependent acquisition of MS/MS full scan spectra for identification. Two MRM transitions were monitored and at the same time EPI spectra were recorded when a signal exceeds a compound specific threshold. The setup of this methods is described in a separate note.[10] The thresholds of the internal standards were set very high to exclude them from MS/MS acquisition.

Results and Discussion

Optimization of LC Conditions

The column dimension 100 x 4.6 mm allowed large volume direct injection without the breakthrough of polar compounds like morphine. The void time of the column was approximately 1 minute, and the elution window of the analytes was between 3.4 and 6.8 minutes.

The 5 µm core-shell material resulted in very sharp chromatographic peaks of approximately 4 seconds width (Figure 1) while the column pressure was very low, ~100 bar, at 900 µL/min and 30°C.

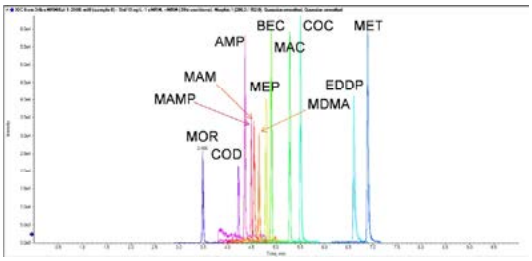

Figure 1. Example chromatogram of a 10 ng/L standard

The optimization of the LC conditions lead to intensity gains up to a factor of 10 for early eluting compounds like morphine and a factor of 2 for medium and late eluting compounds like MDMA (Figure 2). Signal-to-Noise (S/N) values were increased by a factor of 2 to 10.

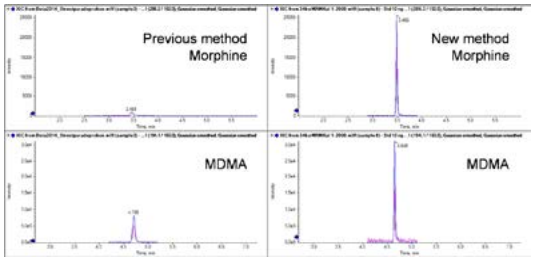

Figure 2. Sensitivity gains for morphine and MDMA by optimizing LC conditions, previous method (left) and newly optimized method (right)

Detuning of Collision Energies (CE)

With sharper and higher LC signals some compounds (e.g. MDMA, benzoylecgonine, cocaine) could cause detector saturation when present at higher concentration in water samples. Some high sensitivity transitions were detuned to minimize this effect and maintain linear dynamic range for quantitation.

The example shown in Figure 3 shows the detuning of CE for benzoylecgonine.

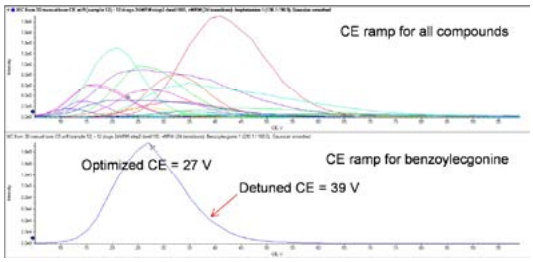

Figure 3. Detuning of CE to minimize detector saturation for benzoylecgonine

Method Data

Method data are listed in Table 4 and summarized below.

Table 4. Sensitivity (S/N calculated using 3x standard deviation in PeakView® software), repeatability (coefficient of variation, %CV) and linearity from 1 to 1000 ng/L (linear fit with 1/x weighting, coefficient of regression, r, using the *Scheduled* MRM™ and the *Scheduled* MRM™-IDA-MS/MS method)

Compound	S/N at 1 ng/L	%CV at 10 ng/L	r (MRM)	r (IDA)
Morphine 1	35	2.5	0.999	0.994
Morphine 2	22	1.4	0.999	0.991
Codeine 1	33	4.4	0.999	0.998
Codeine 2	14	7.1	0.999	0.993
Amphetamine 1	4	4.2	0.997	0.996
Amphetamine 2	8	5.5	0.999	0.997
Monoacetylmorphine 1	14	11.4	0.996	0.992
Monoacetylmorphine 2	15	9.3	0.999	0.995
Methamphetamine 1	3	6.4	0.995	0.994
Methamphetamine 2	17	4.8	0.995	0.993
MDMA 1	24	4.3	0.999	0.993
MDMA 2	2.5	7.7	0.997	0.998
Mephedrone 1	28	6.6	1.000	0.997
Mephedrone 2	14	4.4	0.999	0.994
Benzoylecgonine 1	37	5.9	0.999	0.996
Benzoylecgonine 2	18	5.9	0.999	0.997
Monoacetylcodeine 1	62	4.9	0.998	0.997
Monoacetylcodeine 2	7	4.2	0.998	0.996
Cocaine 1	72	1.9	0.999	0.996
Cocaine 2	15	3.1	1.000	0.998
EDDP 1	47	2.7	0.998	0.998
EDDP 2	43	2.7	0.999	0.998
Methadone 1	43	3.5	0.996	0.985
Methadone 2	22	2.7	0.996	0.989

- Linearity and working range: 1 ng/L to 1000 ng/L for all compounds (except amphetamine and methamphetamine). This corresponds to 2-2000 ng/L for 1:1 diluted samples, and 10-10000 ng/L for 1:10 diluted samples.

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- Limits of quantitation (LOQ): 1 ng/L; S/N > 10 for all compounds (except amphetamine.1 S/N=4, methamphetamine.1 S/N=3)
- Linearity: r > 0.994
- Accuracy of the standards from 1 to 1000 ng/L: between 80 and 120%
- Precision: RSD% typically between 2.5 and 6% for 6 consecutive injections of a standard at 10 ng/L (except acetylmorphine)

Ion suppression

Matrix effects have been investigated by T-infusion experiments. Matrix load of wastewater samples can strongly differ from sample to sample. Generally dilution is necessary to minimize ion suppression when large volume direct injection is used. Figure 4 shows that some suppression effects can still be observed in the elution window of the analytes with a 1:1 dilution. But with dilution 1:10 nearly no ion suppression was observed.

It can also be seen that strong matrix effects are present in the range of the void time up to a retention time of 2.0 min. However, the earliest eluting compound morphine has a retention time of 3.4 min and is not affected by ion suppression.

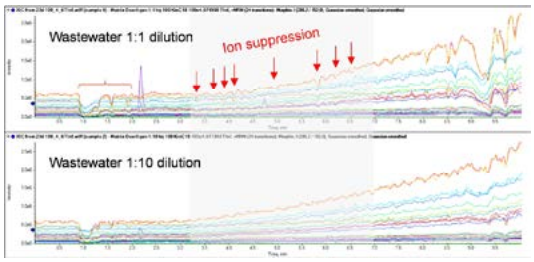

Figure 4. Investigation of matrix effects

Street Parade Results

The gain in sensitivity and the lower detection limits help to detect low levels of illegal drugs. Figure 5 shows the day 5 sample of the Street Parade 2014, diluted 1:10.

Figure 6 shows the profile of MDMA (ecstasy), benzoylecgonine (metabolite of cocaine) and monoacetylmorphine (metabolite of heroin) over the time period of the Street Parade. These profiles indicate different consumption amounts during the event resulting in different wastewater profiles.

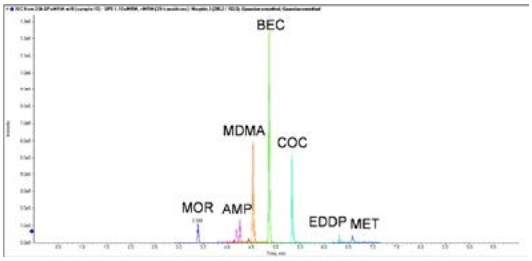

Figure 5. Day 5 sample of the Street Parade Zürich, injected directly after 1:10 dilution

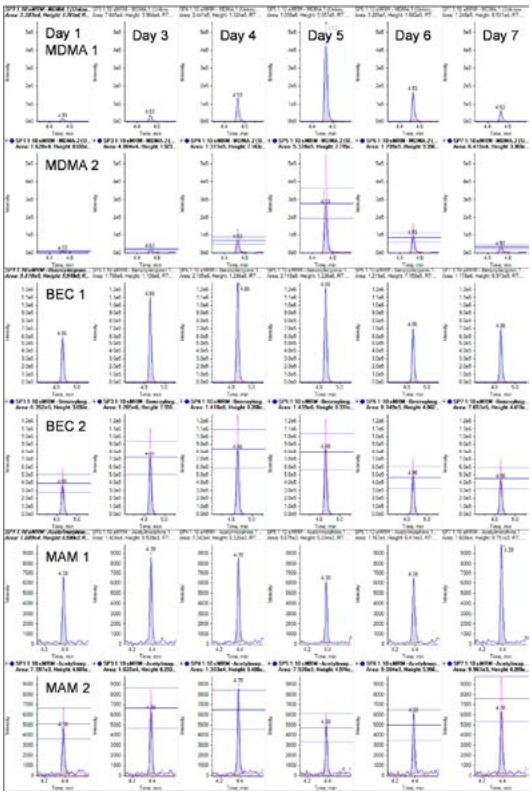

Figure 6. Profile of MDMA (ecstasy), benzoylecgonine (metabolite of cocaine) and monoacetylmorphine (metabolite of heroin) over the time period of the Street Parade. Data indicate high consumption of MDMA and increased consumption of cocaine during the event. The concentration of MAM in wastewater was relatively constant. The peak review in MultiQuant™ software allows reviewing MRM ratios and tolerance levels for compound identification.

Compound Identification

Commonly at least two MRM transitions are monitored per compound and the ratio of quantifier and qualifier ion is used for compound identification. Guidelines define identification criteria and tolerance levels for ion ratios.[11] However, MRM ratios are not always unambiguous. Matrix interferences might disturb one of the two transitions, and thus, ion ratio identification fails. In addition, ion ratios are often falsified at the upper end of the linear dynamic range because of detector saturation. On the other hand, the qualifier MRM can be too weak to be used for identification at the lower end of the dynamic range.

With the *Scheduled* MRM™ pro-IDA-MS/MS workflow it is possible to monitor two transitions for each compound and use the ratio for identification. In addition, QTRAP® MS/MS full scan spectra are collected automatically. These spectra can be searched against mass spectral libraries in MasterView™ software for increased confidence in identification.

The IDA triggering works effectively due to individual thresholds for each compound. Figure 7 shows that chromatograms acquired in IDA mode have slightly less data points across the LC peak at the time where the MS/MS spectrum was acquired, but still enough data points for accurate and reproducible quantitation. Results presented in Table 4 also show that linearity was not compromised when using the IDA method.

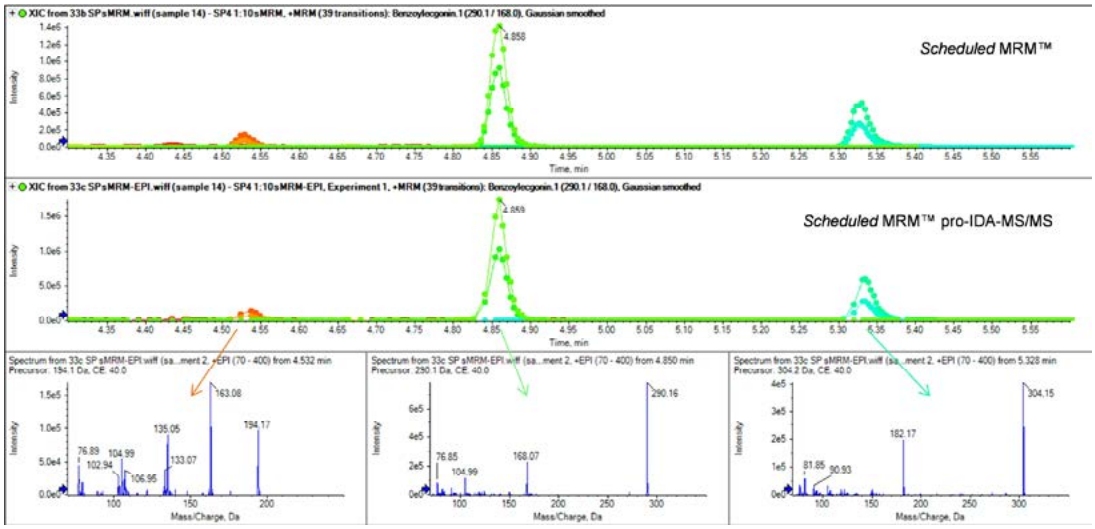

Figure 7. Day 4 sample of the Street Parade Zürich, quantitative data quality is not compromised when using *Scheduled* MRM™ or *Scheduled* MRM™ pro-IDA-MS/MS since sufficient number of data points is acquired using both workflows (top and middle), the IDA methods provides additional information for compound identification (bottom left to right: MS/MS of MDMA, benzoylecgonine, and cocaine)

Standard samples were injected to find out at what concentration compounds can be identified using retention time matching and MS/MS library searching. At 1 ng/L, 9 of the 12 drugs could be clearly identified (no MS/MS spectra were acquired for codeine, amphetamine and monoacetylmorphine) at 5 ng/L, all of the 12 compounds were identified with high confidence (Figure 8).

Figure 9 shows the day 7 sample, diluted 1:10 prior LC-MS/MS analysis. MDMA was identified with high confidence, although the concentration in the injected sample was only 7 ng/L, which corresponds to 70 ng/L in the undiluted sample.

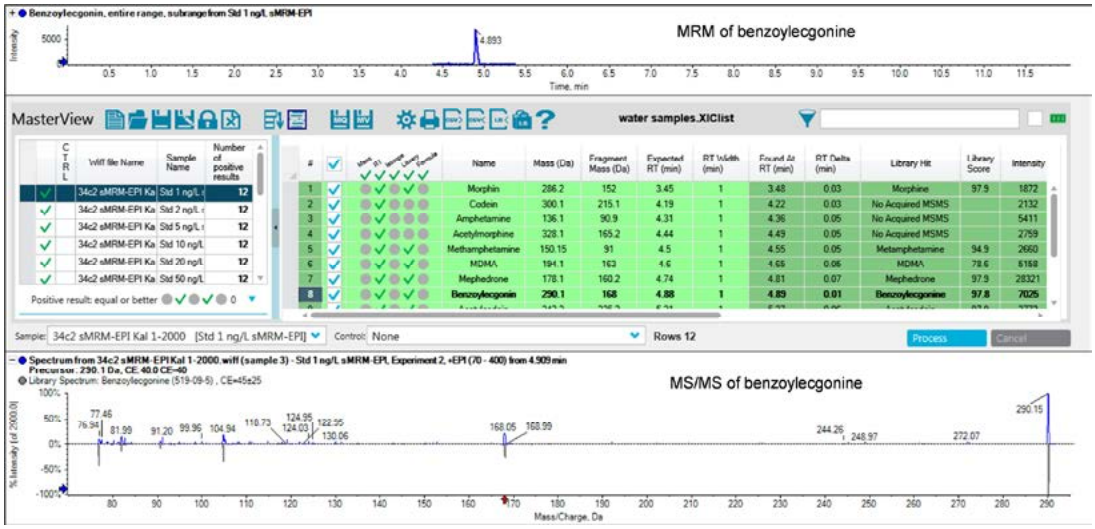

Figure 8. Identification based on retention time matching and MS/MS library searching in MasterView™ software, 9 out of 12 compounds were identified at 1 ng/L, all 12 compounds were identified at 5 ng/L, the example shows the MRM (retention time error = 0.01 min) and MS/MS spectrum (FIT = 97.8%) of benzoylecgonine at a concentration of 1 ng/L

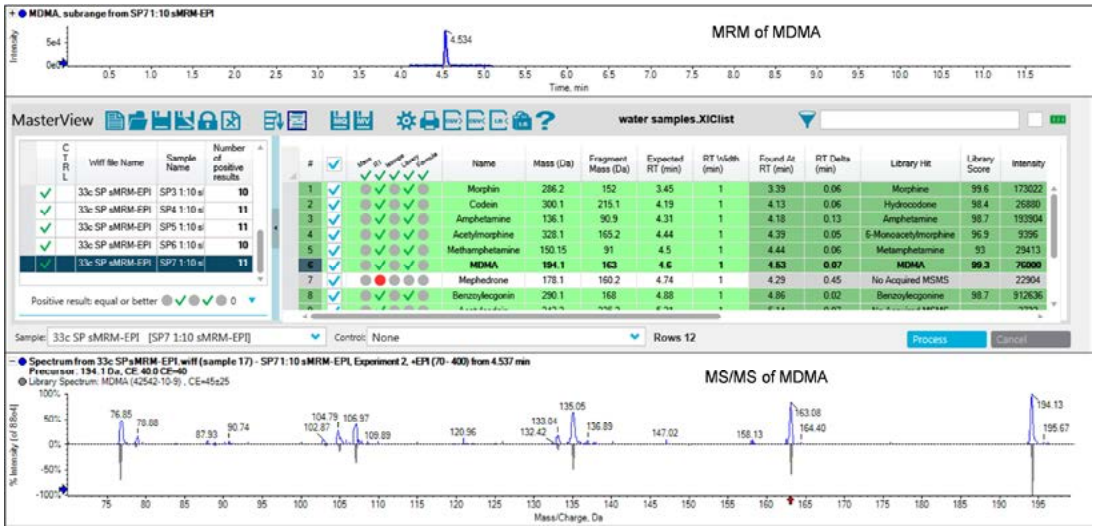

Figure 9. Identification based on retention time matching and MS/MS library searching in MasterView™ software, MDMA was identified with a retention time error of 0.07 min and a library FIT of 99.3%

Contents

Summary

The existing method for the determination of drugs of abuse in complex matrices like wastewater by large volume direct injection was significantly improved. Sensitivity gains and S/N gains by a factor of up to 10 were obtained by optimizing the LC and ion source conditions. This was achieved by using a Phenomenex Kinetex core-shell column with 4.6 mm ID and a high-flow method design. The column backpressure was only ~100 bar due to the use of 5 µm particles and therefore, the method can be run on a traditional LC systems.

The collision energies of high abundant MRM transitions were detuned to avoid detector saturation. Two method workflows were developed using *Scheduled* MRM™ and *Scheduled* MRM™ pro-IDA-MS/MS. Both method allow accurate and reproducible quantitation down to low ng/L range and identification based on ion ratios. The IDA method offers the additional benefit of identifying target analytes based on MS/MS library searching resulting in increased confidence in results.

The method was successfully applied to the determination of drugs of abuse wastewater during a party event (Street Parade Zürich 2014). Different profiles were observed for different drugs indicating different consumption during the time period of the event.

Acknowledgement

The authors wish to thank the people of the Sewage Treatment Plant Werdhölzli, Zurich for the daily wastewater sampling during the Street Parade.

References

1 R.Pal, M.Megharaj, K.P.Kirkbride and R.Naidu: 'Illicit drugs and the environment - a review' Science of the Total Environment 463 (2013) 1079-1092

2 European Monitoring Centre for Drugs and Drug Addiction, Lisbon http://www.emcdda.europa.eu/

3 Chr. Ort, A.L.N. van Nuijs, J.D. Berset et al.: 'Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis' Addiction 109 (2014) 1338-1352

4 S. Castiglioni, K.V. Thomas, B. Kasprzyk-Hordern, L. Vandam and P. Griffiths: 'Testing wastewater to detect illicit drugs: State of the art, potential and research needs' Science of the Total Environment 487 (2014) 613-620

5 D.R. Baker, L. Barron and B. Kasprzyk-Hordern: 'Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A : chemical analysis and drug use estimates' Science of The Total Environment 487 (2014) 629-641

6 J.D. Berset and A. Schreiber: 'Determination of Selected Illegal Drugs and its Important Metabolites in Waste Water by Large Volume Direct Injection HPLC-MS/MS' Application Note AB Sciex (2010) # 0990310-01

7 J.D. Berset, R.Brenneisen and Chr. Mathieu: 'Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-MS/MS)' Chemosphere 81 (2010) 859-866

8 S. Dulaurent, J.M. Gaulier, L. Imbert, A. Morla and G. Lachâtre: 'Simultaneous determination of Δ9-tetrahydrocannabinol, cannabidiol, cannabinol and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in hair using liquid chromatography–tandem mass spectrometry' Forensic Science International 236 (2014) 151-156

9 A. Schreiber and R. Kern: 'Quantitation and Identification of Pharmaceuticals and Personal Care Products (PPCP) in Water Samples' Application Note AB Sciex (2010) # 1790210-01

10 'Using Your QTRAP® LC/MS/MS System at Full Potential - A Quick-Start Guide to Upgrade an MRM Acquisition Method to a Scheduled MRM Pro-IDA-MS/MS Acquisition Method in 6 Easy Steps using Analyst® Software' Application Note AB Sciex (2014) # 10160214-01

11 European Guideline 2002/657/EC

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High Resolution Quantitation of Microcystins and Nodularin on SCIEX X500R QTOF System

KC Hyland, April Quinn-Paquet, Craig Butt
SCIEX, USA

Toxic products of cyanobacteria and aquatic microfaunal communities include the implicated human carcinogens microcystins and nodularins. Microcystins represent an important contaminant class for environmental and drinking water analysis under the US EPA UCMR4 list, effective starting in 2018.

Analysis of microcystins and many other water contaminants is commonly conducted on triple quadrupole systems for sensitivity and robustness. Environmental testing labs are frequently confronted with the need for more confirmatory information when it comes to analyte identification in real world samples. To this end, there has been increased interest and adoption of high resolution/accurate mass (HRAM) systems. Advantages gained include the ability to garner more specificity and qualitative confirmation of compound identification, while maintaining the capacity for sensitive and robust, routine quantitation (Figure 1).

Methods and data are presented which demonstrate use of HRAM for accurate and sensitive quantitation of microcystins and nodularin in water samples.

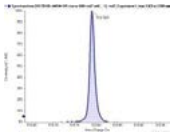
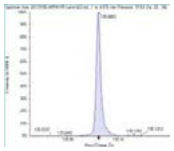
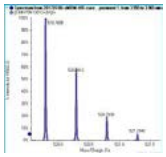
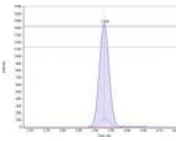
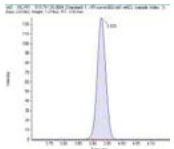
Accurate mass of target precursor		*TOFMS spectrum of MC-RR* *Resolution: 46,000 at 519.8 m/z* *Mass error: <1 ppm* ✓
MRM transition and accurate mass of fragment		*TOFMSMS spectrum of MC-RR* *Precursor: 519.8 m/z* *Fragment Mass error: < 1 ppm* ✓
Isotope matching to theoretical target formula		*Isotope ratio difference <4% MC-RR,* $[C_{49}H_{75}N_{13}O_{12}+2H]^{+2}$ ✓
Ion ratio of TOF MS peak to MRM^{HR} peak match to standard		*Ion ratio percent difference <6%* ✓
Retention time match between standard and unknown		*Chromatographic peak eluting at 3.9min is <1% difference from expected RT* ✓

Figure 1. Target Identification Points of Confirmation. Identification and quantitation of microcystins in unknown samples can be achieved with high confidence using HRAM analysis due to multiple points of matching using accurate mass, MRM^{HR}, isotope pattern matching, ion ratio, and chromatographic retention time.

Key Assay Attributes

- Quantitation of a suite of eight microcystins (MC) and one nodularin (NOD) was achieved using high resolution accurate mass LC-MS/MS with the SCIEX X500R QTOF system.
- Utilizing data acquisition with high resolution Multiple Reaction Monitoring (MRM^{HR} workflow) in conjunction with the simultaneous collection of TOF MS data, multiple approaches to achieving accurate and sensitive quantitative analyses for these analytes in drinking water matrices are presented.
- Sensitivity was demonstrated by LOQ values ranging from <0.1µg/L - 3µg/L.
  - US EPA 10-day drinking water health advisory for microcystins is 0.3 µg/L for infants and children up to 6 years old, and 1.6 µg/L for adults.
- Confidence in compound identification is imparted by the specificity of accurate mass data for the ionized compound in TOF MS mode, as well as the product ion generated from the isolated precursor in MRM^{HR} workflow (Figure 1).

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Experimental

Standards: Neat standards were obtained from Enzo Life Sciences (Farmingdale, NY) and reconstituted in 1 mL of methanol. An intermediate mixed stock was prepared by diluting the standards in methanol to yield 500 µg/L for MC-RR and Nodularin-R, and 2000 µg/L for MC-LA, MC-LF, MC-LR, MC-LY, MC-LW, MC-YR, MC-WR. Calibration standards were prepared with 5% acetonitrile in water to match the initial LC conditions. Standards were prepared in glass vials and kept at -20°C until analysis.

HPLC System: A SCIEX ExionLC™ AD was used as the LC system. Chromatographic separation was achieved under gradient conditions using a Phenomenex Kinetex® C8 column (2.6 µm particle size, 100 x 3 mm) and flow rate of 0.500 mL/min. The mobile phases were water with 0.1% formic acid ("A") and acetonitrile with 0.1% formic acid ("B"). Column oven temperature was 40°C and a 20 µL injection was used. To reduce sample carryover the autosampler rinse solvent was 60:20:20 isopropyl alcohol: methanol: acetonitrile using a rinse volume of 2 mL and dip time of 8 s. Run time was 11 minutes for the full gradient. An example elution profile is shown in Figure 2.

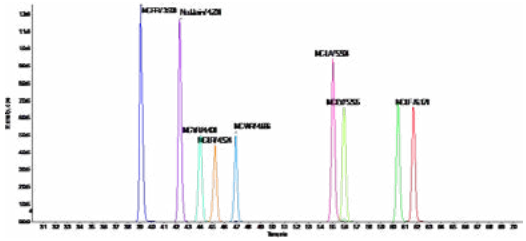

Figure 2. Chromatographic Separation of 8 Microcystins and 1 Nodularin. Separation was achieved using a SCIEX ExionLC AD system and a Phenomenex Kinetex C8 column over an 11-minute gradient with water and acetonitrile.

TOF MS/MS Parameters and Detection: The SCIEX X500R QTOF system with the Turbo V™ source was operated in positive mode electrospray ionization (ESI). Source parameters are listed in Table 1. The TOF MS scan was conducted over a range of 500 to 1100 m/z. MRM^{HR} experiment included two transitions monitored for each analyte. For each transition in the acquisition method, the precursor ion was defined for the target analyte, and a mass range was defined which would encompass the expected fragment ion. Optimized declustering potential (DP) and collision energy (CE) voltages were designated for each transition, based on optimized values determined from the Guided MRM^{HR} algorithm. Additionally, retention time (RT) values were specified for each MRM^{HR} transition, with RT tolerance values of 15 s for each.

Table 1. Ion Source Parameters. Electrospray Ionization (ESI) was conducted in positive ion mode.

Parameter	Setting
Curtain Gas (CUR)	*30*
Collision Gas	*10*
Ion Spray voltage (IS)	*5500*
Temperature (TEM)	*650*
Nebulizer Gas (GS1)	*50*
Heater Gas (GS2)	*50*

Data Analysis and Method Performance Evaluation: Data acquisition and processing were performed using SCIEX OS software. Calibration curves were generated from the injection of seven concentration levels from 0.12 to 312 µg/L. Linear regression models with 1/x weighting were used to fit all calibration curves, and 1-point Gaussian smooth was applied to data for calibration and sensitivity evaluation. Example calibration curves for a subset of analytes can be seen in Figure 3. For the evaluation of method sensitivity, the limit of detection (LOD) was defined as the lowest calibrator with peak-to-peak signal to noise ≥3. The limit of quantitation (LOQ) was defined as the lowest calibrator with peak-to-peak signal to noise ≥10.

Table 2. Accurate Masses for Microcystins and Nodularin. The formula and accurate monoisotopic m/z for each analyte studied is provided.

Compound ID	Formula	Adduct	Monoisotopic m/z
MC-RR	$C_{49}H_{75}N_{13}O_{12}$	[M+2H]++	519.79018
Nodularin	$C_{41}H_{60}N_8O_{10}$	[M+H]+	825.45052
MC-LA	$C_{47}H_{68}N_6O_{12}$	[M+H]+	909.49680
MC-LF	$C_{52}H_{71}N_7O_{12}$	[M+H]+	986.52335
MC-LR	$C_{49}H_{74}N_{10}O_{12}$	[M+H]+	995.55604
MC-LY	$C_{52}H_{71}N_7O_{13}$	[M+H]+	1002.51826
MC-LW	$C_{54}H_{72}N_8O_{12}$	[M+H]+	1025.53425
MC-YR	$C_{52}H_{72}N_{10}O_{13}$	[M+H]+	1045.53531
MC-WR	$C_{54}H_{73}N_{11}O_{12}$	[M+H]+	1068.55129

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Key Quantitation Limits for Assay

US EPA 10-day drinking water health advisory for microcystins is 0.3 µg/L for infants and children up to 6 years old, and 1.6 µg/L for adults. Maximum acceptable concentration (MAC) of MC-LR is 1.5 µg/L in Canada, and the World Health Organization (WHO) MC-LR provisional guideline is 1 µg/L. The EPA additionally has included several of these compounds of its Fourth Unregulated Contaminant Monitoring Rule (UCMR4). Minimum Reporting Levels (MRLs) ranging from 0.005 to 0.09 µg/L are listed in this document based on EPA standard Method 544. EPA Method 544 utilizes LC-MS/MS with up front concentration of water samples using solid phase extraction (SPE).

Quantitation Results

Quantitation was achieved by utilizing high resolution TOF MS data of each precursor. MRM^{HR} transitions were acquired and used for quantitation and confirmation. Both scans occur within every cycle during an injection, and processing of the acquired data can utilize either or both. Comparison of these two quantitative analyses indicate that quantitation of these microcystins using the TOF MS extracted accurate mass is sensitive, linear, and precise; and demonstrates better performance compared to MRM^{HR} for quantitation. TOF MS data was processed using known analyte charged monoisotopic masses (*m/z*) and a mass extraction width (XIC width) of 0.02 Da. Table 2 shows the extracted precursor masses used. Microcystins in the analyzed suite have MW >900. Ionization was performed in positive ion mode, but it is noted that the target MCs will protonate to form [M+H]+ species as well as doubly charged species. Eight of the nine analyzed targets show the singly charged species as dominant; it was therefore used for monitoring and quantitation. However, MC-RR showed the +2 charge as a greater intensity precursor than [M+H]+. The *m/z* values monitored for this precursor reflect the choice of the [M+2H]++ species for maximized sensitivity (Table 2).

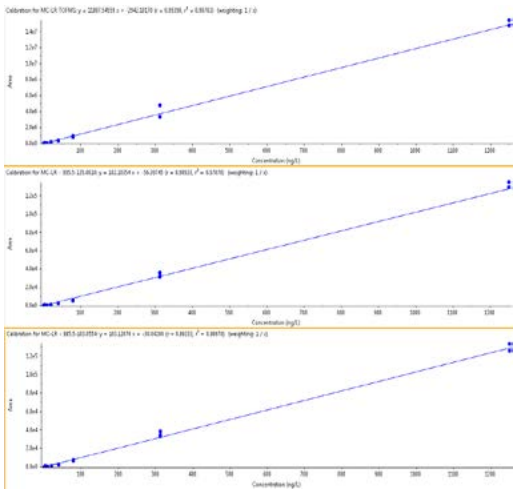

Figure 3. Calibration Curves for MC-LR. Curves are shown for one of the target analytes, MC-LR, for three different acquisition channels. The isolation of the TOF MS accurate mass (top), as well as the two optimized MRM^{HR} transitions, can all be detected in calibration standards to produce a regression for quantitation.

The 11-minute chromatographic gradient with the Kinetex C8 column achieved baseline separation for all compounds (Figure 2). The gradient is 15 min shorter than the program described in EPA Method 544, resulting in considerable time savings while maintaining separation and peak quality.

The LOD and LOQ concentrations for all compounds were demonstrated to be well below the lower range of the calibration curve (lowest calibrator 0.12 µg/L) when analyzing using TOF MS data (Table 3). Signal- to- Noise ratios at 0.12 µg/L ranged from 27 (for MC-LR) to 91 (for MC-RR) demonstrating that lower concentrations may be detected and quantified using this method. Precision is shown as the %CV of triplicate analyses and is also listed in Table 4 for two concentration levels. Reproducibility is excellent with all %CV values of 7% or less for the TOF MS data. Linear dynamic range was also calculated and shown to be between 2.5 and 3.4 orders of magnitude. MRM^{HR} data showed LOQ concentrations in the range of 1 – 3 µg/L. Precision at the low levels is also reduced compared to the TOF data, likely as a result of the decrease in the absolute signal available in the MS/MS data.

A key advantage of HRAM approach to quantitation is the ability to apply multiple points of confirmation to avoid reporting false positive detection (Figure 1). The five points of confirmation applied in this method are:

1. Accurate mass of the target precursor
2. Confirmation with the MRM^{HR} transition and the accurate mass of the fragment ion
3. Isotope ratio matching to theoretical pattern based on target formula
4. Ion ratio matching between standard and unknown sample
5. Retention time matching between standard and sample

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Table 3. Quantitative Analysis Method Performance. Detection and quantitation limits, linearity, and reproducibility for the microcystins suite. TOF MS data processed with a mass extraction width (XIC width) of 0.02 Da.

Compound ID	LOD (µg/L)	S/N at 0.12µg/L	%CV at 0.12µg/L	%CV at 4.88ppb	Cal Range (µg/L)	Curve fit, Weighting	Dynamic Range (log[ULOQ/LLOQ])
MC-RR	<0.12	91	7%	4%	0.12 – 312.5	Linear, 1/x	3.4
Nodularin	<0.12	60	2%	3%	0.12 – 312.5	Linear, 1/x	3.4
MC-LA	<0.12	39	2%	5%	0.12 – 312.5	Linear, 1/x	3.4
MC-LF	<0.12	36	2%	2%	0.12 – 312.5	Linear, 1/x	3.4
MC-LR	<0.12	27	2%	4%	0.12 – 78	Linear, 1/x	2.8
MC-LY	<0.12	32	2%	3%	0.12 – 78	Linear, 1/x	2.8
MC-LW	<0.12	31	2%	2%	0.12 – 78	Linear, 1/x	2.8
MC-YR	<0.12	17	2%	2%	0.12 – 312.5	Linear, 1/x	3.4
MC-WR	<0.12	15	2%	1%	0.12 – 39	Linear, 1/x	2.5

High MS Data Quality for Confirmation

High resolution TOF MS analysis of microcystins shows mass resolution >40,000 across the range of precursor m/z. This mass resolution infers a high degree of specificity for the target analyte. Mass accuracy within 2ppm reinforces confidence in the identification of the targets. High resolution fragments collected using MRM^{HR} also demonstrate excellent specificity and confidence from high mass resolution and mass accuracy (Figure 4 and 5). TOF MS pattern of resolved MS isotopes was compared to the theoretical isotope pattern for the empirical formulae of each microcystin; these data provide additional confirmation of the presence of the compound and are displayed in the results table within the SCIEX OS software. Precursor XIC and MRM^{HR} transition data can also be grouped together in the data processing module of SCIEX OS, so that peak data corresponding to the same compound are processed as such; this allows the software to automatically evaluate ion ratios by comparing signal from multiple acquisition channels for a single analyte. Ion ratio comparison of sample data to that of standards provides another metric for qualitative matching and identity confirmation.

Summary

Quantitation of a suite of microcystins and nodularin was demonstrated using LC-MS/MS on SCIEX X500R QTOF system. Utilizing the TOF MS spectral data and extracting a narrow XIC for analyte precursor ions provided excellent quantitation results with sensitive detection (<0.1µg/L LOD) and exceptional reproducibility (%CV <7%) at low concentrations. If used with the EPA Method 544 suggested concentration factor of 500-fold, these detection limits are theoretically extended to 0.0002 µg/L, attaining the MRLs defined in the UCMR4 list. Linearity was reported over 5-6 concentration levels and approximately three orders of magnitude with r-values from 0.95 to 0.99. MRM^{HR} data also provided calibration and quantitation information, with sensitivity down to 1 – 3 µg/L.

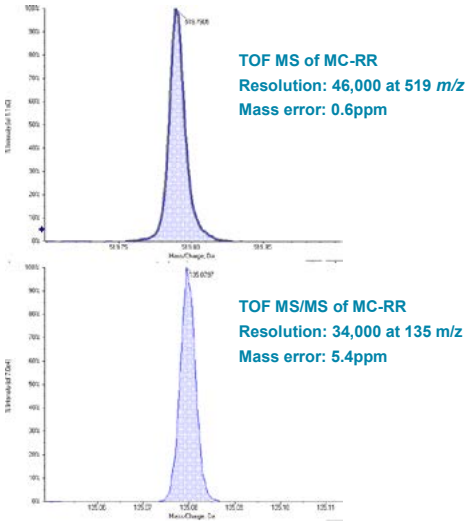

Figure 4. HRAM TOF MS Analysis of Microcystin MC-RR. Mass spectral data shows high mass resolution and mass accuracy, providing specificity for the target analyte. Mass accuracy within 2ppm for the precursor for the TOF MS precursor reinforces confidence in the identification of the targets. MRM^{HR} fragments also demonstrate excellent specificity and confidence with resolution >28,000 and mass error <6ppm.

Concurrent MRM^{HR} data allows for additional quantitative information and enhanced confidence in qualitative analysis without requiring additional injections or processing. Using the HRAM approach on the X500R system, confirmation of target identification can be attained using accurate precursor mass matching, isotope pattern matching, accurate fragment mass matching, ion ratio matching, and retention time matching. Verifying across these five points reinforces the ability to confirm the presence of the analyte and helps safeguard against reporting false positives.

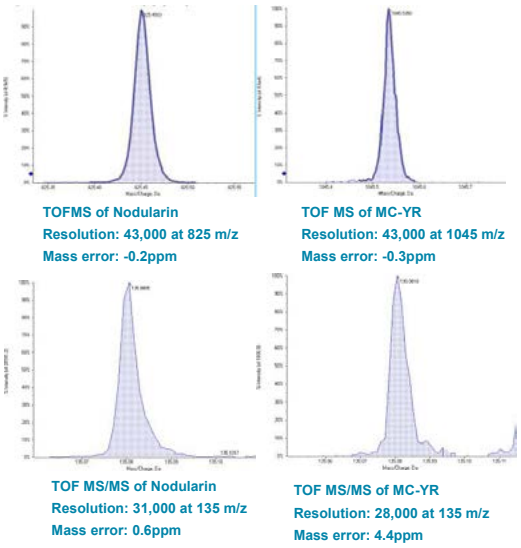

Figure 5. HRAM TOF MS Analysis of Microcystin MC-YR and Nodularin. Mass spectral data shows high mass resolution and mass accuracy, providing specificity for these target analytes. Mass accuracy within 2ppm for the precursor for the TOF MS precursor reinforces confidence in the identification of the targets. MRM^{HR} fragments also demonstrate excellent specificity and confidence with resolution >28,000 and mass error <5ppm.

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Rapid Characterization of Naphthenic Acids Using High Resolution Accurate Mass MS and MS/MS with SelexION™ Differential Mobility Separation

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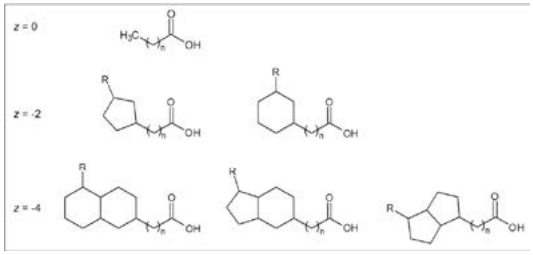
Overview

This study demonstrates the rapid gas-phase analysis of naphthenic acids using the AB SCIEX TripleTOF® 5600+ system with SelexION™ differential mobility separation. The assets of this workflow include:

1. The rapid characterization (< 2 min) of naphthenic acids in complex samples.
2. Utilizing SelexION™ to isolate individual naphthenic acids on-demand (including isomers and isobars) for in-depth structural analysis.
3. Accounting for background ions using their SelexION™ mobility.

Introduction

Naphthenic acids (NA) from oil sands process-affected water (OSPW) have been the subject of numerous mass spectrometry-based environmental studies.[1, 2]



The classical definition of these compounds ($C_nH_{2n+z}O_2$, where z is an even negative integer representing hydrogen deficiency) has recently been expanded to the naphthenic acid fraction component (NAFC), which includes unsaturated and aromatic NA derivatives, increased oxygen content and compounds containing nitrogen and/or sulfur.[1-3]

NAFCs are of particular concern in northern Alberta, Canada, where the caustic extraction of bitumen from surface mineable oil sands produces large volumes of OSPW.[4]

A common workflow for NAFC analysis involves direct infusion into an ultrahigh resolution mass spectrometer. A resolving power >100,000 is critical to obtaining useful NAFC profiles with this approach. While such instruments can provide elemental compositions, additional steps are required to interrogate molecular structure. To overcome this, chromatography is often utilized. Unfortunately, the high complexity of OSPW extracts may necessitate relatively long run times, multiple sample handling steps, or multi-dimensional chromatography. Moreover, traditional chromatography is an inherently serial process, with limited time available to perform deeper structural interrogation of individual analytes.

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The AB SCIEX TripleTOF® 5600+ high resolution accurate mass system coupled with SelexION™ differential mobility spectrometry presents a unique workflow for NAFC analysis.[5]

The combination of gas-phase separation and the ability to access analytes on-demand via direct-infusion resolves NAFCs from OSPW extracts rapidly (< 2 min), generating complex and insightful datasets. Such rapid, information rich methods will become important as regulatory guidelines for NAFC analyses are implemented and testing laboratories have to accommodate increased demand.

Experimental

A technical NA mix was obtained from the Merichem Company (Houston, TX) and an OSPW extract was obtained by extracting a sample from an industrial location in the Athabasca River Basin, Alberta, Canada.[6]

Analyses were conducted using the AB SCIEX TripleTOF® 5600+ system with SelexION™ or the AB SCIEX QTRAP® 5500 system with SelexION™. The operating parameters are listed in Table 1.

Table 1. Typical SelexION™ and TripleTOF® operating parameters

Parameter	Value
Infusion rate	15 µL/min
IonSpray voltage	-4500 V
Source temperature	100°C
Gas 1	20 psi
Gas 2	10 psi
Modifier composition	1.5% (v/v)
SelexION™ temperature	150°C
Separation Voltage (SV)	4000 V_{pp}
Compensation Voltage (CoV)	-17 to +20 V (0.25 V steps)
TOF m/z range	50 - 2000
TOF accumulation time	250 msec
Declustering Potential (DP)	-100 V
MS/MS Collision Energy (CE)	-35 to -50 V

For the SelexION™ experiments two operational modes were employed:

1. Separation voltage was held at an optimum value while the compensation voltage was scanned; and
2. At each CoV increment, MS or MS/MS spectra were recorded.

Data were plotted as ionograms (Signal Intensity versus CoV).

Data acquisition was carried out using Analyst® TF software and data was interrogated using PeakView® software version 2.0 and an accurate mass database (8,127 entries) with the following parameters: $C_{5\text{-}20}$, z0-16, $O_{2\text{-}8}$, $N_{0\text{-}2}$, $S_{0\text{-}2}$. Results were confined to ±10 ppm of the database masses and were compared to suitable blanks.

Results and Discussion

SelexION™ mobility functions by transmitting ions between two planar electrodes, across which a high-voltage rf asymmetric waveform is applied (Figure 1).

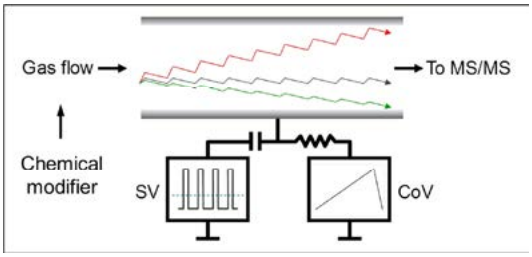

Figure 1. Schematic of the SelexION™ mobility cell

The difference between the ion's mobility during the high- and low-field portions of the waveform determines their SelexION™ mobility. Chemical effects are also critical to ion separation using SelexION™, as the addition of volatile modifiers like methanol to the transport gas can alter ion mobility. To mitigate the complexity of NAFC analysis, a series of chemical modifiers were added to the transport gas and the resulting SelexION™ peak capacities were evaluated. Since each ion was transmitted with a full-width half maximum of ~2.5 V, the greater the spread in total CoV space covered by all of the NAFC ions, the greater the peak capacity of the SelexION™ separation. Among the modifiers examined, methanol yielded the greatest total spread in CoV (Figure 2).

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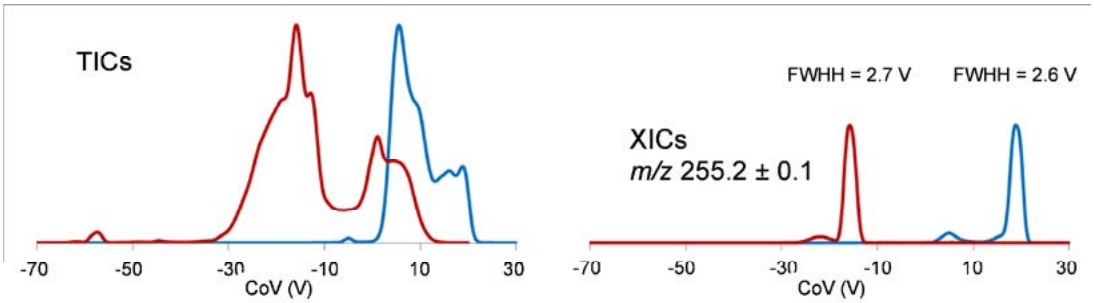

Figure 2. SelexION™ behavior of an OSPW extract with (red) and without (blue) methanol added in the N_2 carrier gas. Extracted ion chromatograms are shown as an example of typical peak widths (right). Signal intensity was normalized for ease of data presentation. FWHM – full width at half maximum height.

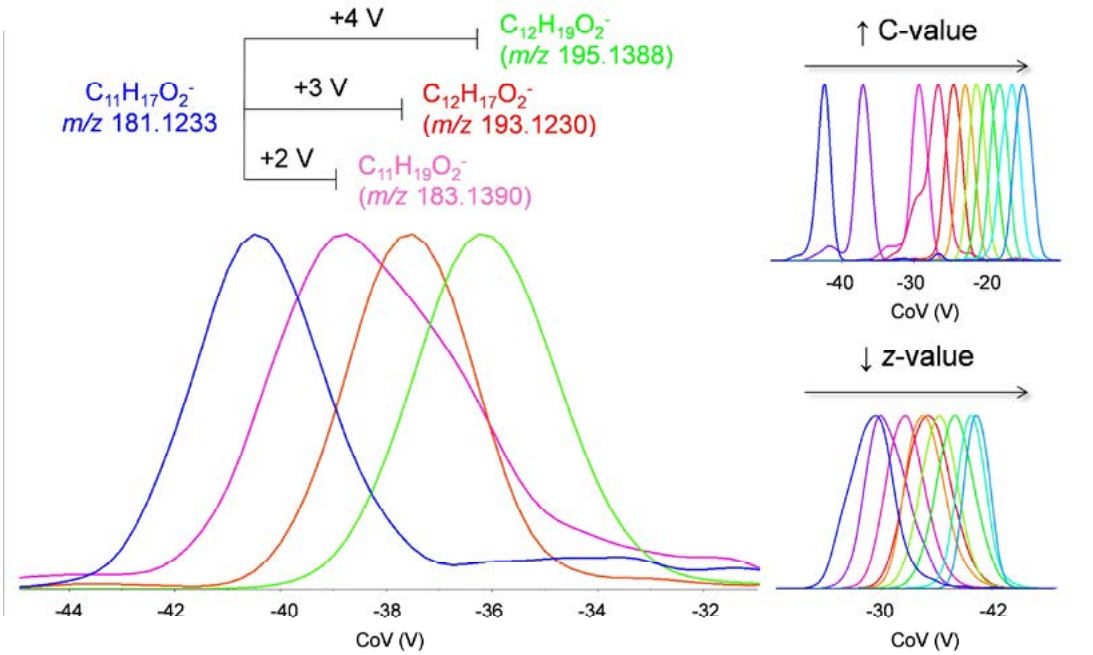

Figure 3. Thorough data analysis demonstrated that SelexION™ behavior was not simply correlated to *m/z* (left). Trends in CoV shifts for carbon number (top right) and z-series (bottom right) are consistent with reported LC behavior of similar samples. Data was normalized to 100% within each ionogram and is shown for an OSPW extract.

The SelexION™ separation of individual NAFC ions suggested a correlation between ion mobility and structure (Figure 3).

For example, *m/z* 181.1233 ($C_{11}H_{17}O_2^-$) was separated from the more saturated analogue at *m/z* 183.1390 ($C_{11}H_{19}O_2^-$, only two hydrogens added) by +2 V. However, this $C_{11}H_{17}O_2^-$ ion was only separated by +3 V from $C_{12}H_{17}O_2^-$ (an acid one carbon atom heavier) and by +4 V separated from $C_{12}H_{19}O_2^-$ (one CH_2 unit heavier).

Clearly, the more unsaturated analogues exhibited more negative CoVs, and the ring/double bond analogues displayed different mobility behavior than the linear chain extended analogues. The fact that such subtle structural differences result in the separation of closely related ions demonstrates the analytical potential of SelexION™-based workflows. In addition to the above observations, correlations between CoV and homologous carbon series and *z*-series emerged (Figure 3).

A Kendrick mass plot (plot of Kendrick mass defect as function of Kendrick mass) provides a high level means of assessing the composition of complex mixtures like OSPW extracts. In the Kendrick mass plot (Figure 4) all relevant mass peaks in a spectrum are normalized against methylene, such that a horizontal line represents a homologous carbon series for a particular compound class. Moreover, as the degree of unsaturation increases, so does the Kendrick mass defect. This translates to easy to assess trends. For instance, in Figure 4 the red lines indicate a series of simple acids (i.e., only O_2 compounds) with increasing *z*-values, while the green lines represent a homologous series of O_3 compounds at differing degrees of unsaturation. Finally, the blue lines demonstrate the observable trends in unsaturation for a given carbon number.

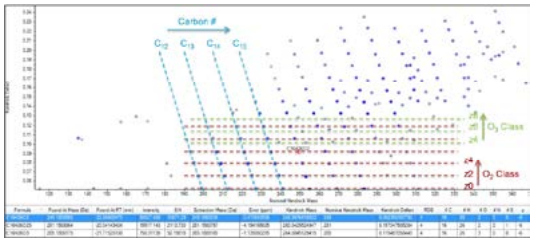

Figure 4. Kendrick mass plot of the OSPW extract generated in an add-in of PeakView™ software

Another interesting attribute of the SelexION™ separation of NAFCs was the gas-phase resolution of isobaric and isomeric ions. For example, in the OSPW extract *m/z* 143.1080, corresponding to $C_8H_{15}O_2^-$ (1.7 ppm), was transmitted though the SelexION™ cell at two CoVs (Figure 5).

The separation of these two potential isomers was rapid (~3 sec) and due to the infusion-based sample analysis, MS/MS analysis of each *m/z* 143.1080 ion was easily performed by fixing the SelexION™ cell at the appropriate CoV value. The resulting MS/MS spectra yielded fragmentation patterns that were consistent with the presence of distinct isomeric species. Analysis of three authentic $C_8H_{16}O_2$ isomers revealed that the OSPW extract contained 2-ethylhexanoic acid and n-octanoic acid (Figure 5). Separation of such isomers by GC or LC generally requires minutes of elution time. Using SelexION™, these isomers were separated in seconds and could be analyzed on-demand.

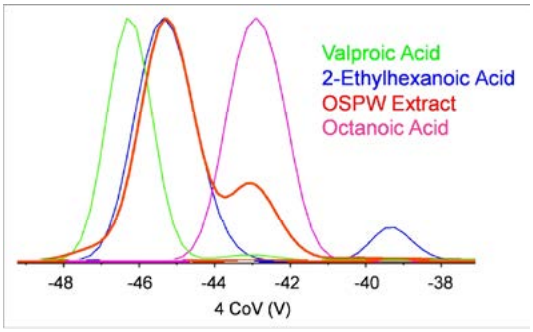

Figure 5. Apparent structural isomers of $C_8H_{16}O_2$ were identified in the OSPW extract (red). Subsequent analysis of valproic acid (green), 2-ethylhexanoic acid (blue) and octanoic acid (pink) standards showed that 2-ethylhexanoic acid and octanoic acid were present in the OSPW extract and partially resolved using SelexION™.

There were also numerous examples where SelexION™ separated isobaric species. In one case, palmitic acid ($C_{16}H_{31}O_2^-$), which is a known contaminant in laboratory environments, was observed at *m/z* 255.2329 (Figure 6).

Alongside the ionized palmitic acid was an isobar at *m/z* 255.1405. TOF-MS can easily resolve these ions, but further interrogation by MS/MS would be complicated without SelexION™ since both ion populations would be sampled by the quadrupole mass filter and a heavily convolved MS/MS spectrum would result. Like the $C_8H_{16}O_2$ isomer separation, selectively tuning the CoV facilitated the interrogation (MS and MS/MS) of each isobar in real-time.

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The anion at *m/z* 255.2329 (CoV = -21 V) fragmented via loss of water (*m/z* 237.2234, 4.3 ppm) as the dominant product ion, which is consistent with palmitic acid. The MS/MS of *m/z* 255.1405 (CoV = -26 V) showed carbon dioxide loss (*m/z* 211.1520, 2.3 ppm) as the dominant product ion, suggesting a carboxylic acid (Figure 6). The elemental composition $C_{17}H_{19}O_2^-$ correlated with *m/z* 255.1405 (5.7 ppm).

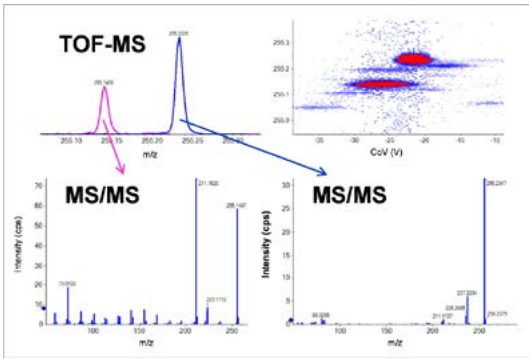

Figure 6. SelexION™ is capable of rapidly resolving isobars from an OSPW extract via mass resolution (top left) and ion mobility (top right). MS/MS analysis of *m/z* 255.2329 is consistent with palmitic acid (bottom right), while the *m/z* 255.1405 is consistent with another carboxylic acid (bottom left).

In LC-MS analyses of NAFCs, interferences like palmitic acid, stearic acid, or dodecyl sulfate, often present as systematic contaminants, are ionized continuously throughout the chromatographic run. These ever-present background ions may deteriorate mass spectrometer performance. SelexION™ efficiently removes background ions by sequestering them in discrete CoV ranges, outside of which they will not appear in the analytical data. In this study, it was critical to obtain an accurate assessment of the potential interfering species present in the analytical samples to ensure that only NAFCs were reported from the database search.

The combination of SelexION™ differential mobility separation and detection using the AB SCIEX TripleTOF® 5600+ revealed 12 background compounds that were present, including saturated and monounsaturated fatty acids, several of which are known background ions in laboratory solvents and analytical instrumentation. One series of interest belonged to the O_3S compound class, with *z* = -6. The series ranged from C_{16} to C_{19} with mass accuracies of 0.5 to 3.0 ppm. MS/MS of these ions after isolation with SelexION™ verified the presence of alkyl-substituted benzylsulfonates (Figure 7).

These detailed structural analyses were quickly and easily facilitated using SelexION™ by tuning the CoV to the appropriate value for each background ion. While this series exhibited relatively low responses, their inclusion in the Merichem and OSPW data would present a false indication of the compound classes present.

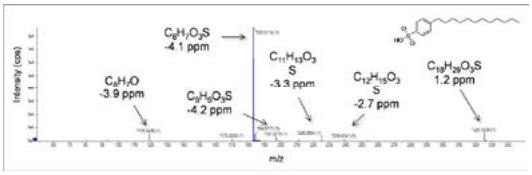

Figure 7. Example MS/MS analysis for the O_3S components identified in the ACN Blank, Merichem standard and OSPW extract. Proposed structure and detailed MS/MS assignments are shown for *m/z* = 325.1839, which corresponds to $C_{18}H_{30}O_3S$ within 1.2 ppm at the MS level and within 3.7 ppm at the MS/MS level. Values shown for product ion assignments are given with calculated mass errors. The product ions at *m/z* 183.0114 and *m/z* 119.0498 are characteristic of alkyl-substituted benzylsulfonates.[7]

All Merichem standard and OSPW extract database matches were compiled as a function of compound class and *z*-value. Results were broken down further based on total response and the number of homologues identified (Figure 8).

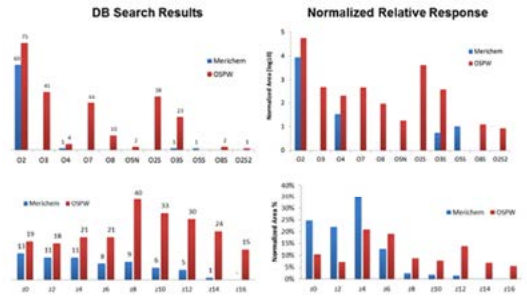

Figure 8. Evaluation of Merichem and OSPW samples as a function of the number of database-identified compounds grouped by class (left) and area response (right). Results are further broken down by compounds class (top) and *z*-value (bottom). Values shown above the homologue plots are the number of homologues identified during database (DB) searching. Due to the large differences in absolute response between compound classes this data was converted to a log10 scale.

Contents

Exact agreement between the distribution of ion classes observed in the current study and literature values was not expected given the heterogeneity of NAFCs.

By area response (99%) and the number of identified homologues (97%), the Merichem standard consisted primarily of O_2 species. Analysis of the Merichem results by *z*-value showed a significant number (42%) of homologues $z \leq -6$ (i.e., -8, -10, etc.). This result seemed contradictory to reported compositions, which state that the Merichem standard consists predominantly of compounds $z \geq -4$.[1, 3] However, these compositions were all response comparisons, not an identification of the number of homologues present. The response data in the current study showed that those compounds $z \geq -4$ accounted for 82% of the total area response, consistent with literature reports. The results by compound class (log10 scale) supported this comparison, with the Merichem standard containing almost exclusively O_2 species and a small amount of higher oxygen content and heteroatom-containing species.

The OSPW extract showed a more widely distributed number of database matches across the compound classes and *z*-values evaluated (Figure 8). Interestingly, despite comprising 49% of the positive DB matches, O_3 to O_8 compounds only accounted for 2.8% of the total area response. Similar to the Merichem results, this suggests that such compounds either have low response factors and/or are present at low levels. Conversely, the *z*-value results for the OSPW extract showed a relatively even distribution across the number of database matches and the relative area responses of the different *z*-classes. These results are consistent with the demonstrated composition of OSPW extracts, which are known to contain higher oxygen content and increased unsaturation/polycyclic compounds due to natural weathering and metabolic processes.[1, 3]

A detailed breakdown of the compound classes (log10 scale) by area response showed that the OSPW extract was composed of mostly O_2-species (86%), with significant amounts of O_2S (6.6%), O_3 (0.8%) and O_3S (0.6%) compounds (Figure 7). In addition, several potentially interesting compound classes (e.g., O_2NS, O_5NS, and $O_4N_2S_2$) were observed at very low abundances (<0.08% of total area).

Summary

The AB SCIEX TripleTOF® 5600+ system with SelexION™ differential mobility separation generates complex and insightful datasets very quickly (< 2 min), including the resolution of isomers and isobars. The ability to scan the SelexION™ cell to target specific analytes provides on-demand access to deeper structural interrogation. With its speed and lower resource cost compared to chromatographic alternatives, SelexION™ represents a greener analytical technique that requires much less organic solvent or gas consumption.

Analysis of a technical Merichem standard and an OSPW extract demonstrated results that are consistent with literature values for similar samples. Owing to the unique separation mechanism of SelexION™, structural isomers can be resolved and rapidly interrogated in real-time. Finally, compared to standard infusion-based NAFC analyses, SelexION™ coupled to the TripleTOF® 5600+ should provide more accurate qualitative and quantitative results owing to the mitigation of background ions and deleterious space charge effects possible when directly infusing complex mixtures.

Acknowledgement

The authors thank Michael McDonell and Lyle Burton (AB SCIEX) for helpful discussions, as well as Carmai Seto and Ashish Pradhan (AB SCIEX) for invaluable assistance with these experiments.

References

1 J.V. Headley et al.: 'Chemical fingerprinting of naphthenic acids and oil sands process waters—A review of analytical methods for environmental samples' J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 48 (2013) 1145-1163

2 J.V. Headley, K.M. Peru, M.P. Barrow: 'Mass spectrometric characterization of naphthenic acids in environmental samples – a review' Mass Spectrom. Rev. 28 (2009) 121-134

3 D.M. Grewer et al. 'Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured?' Sci. Total Environ. 408 (2010) 5997-6010

4 X. Han, M.D. MacKinnon, J.W. Martin: 'Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS' Chemosphere 76 (2009) 63-70

5 M.R. Noestheden et al.: 'Rapid characterization of naphthenic acids using differential mobility spectrometry and mass spectrometry' Environ. Sci. Tech. 48 (2014) 10264-10272

6 V.V. Rogers, K. Liber, M.D. MacKinnon: 'Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water' Chemosphere 48 (2002) 519-527

7 U. Ceglarek et al.: 'Determination of linear alkylbenzenesulfonates in communal wastewater by means of solid phase microextraction coupled with API-MS and HPLC-FLD' Fresenius J Anal Chem 365 (1999) 674-681

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LC-MS/MS Solution for Determination of Nitrosamine Disinfection Byproducts in Drinking Water

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Following the 2016 publication of the article, "Nitrosamine Carcinogen, 'Silent Killer' - Urgent Water Quality Standards," nitrosamine compounds have rapidly drawn the widespread attention of the public and of those in the field of analytical monitoring. N-Nitrosodimethylamine (NDMA) is one of the known byproducts of nitrosamine disinfection and has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer. The World Health Organization (WHO) has established a limit of 100 ng/L NDMA in drinking water, although no such quality standards for limiting nitrosamines in drinking water exist in China. In response to public concern, this document presents the use of the QTRAP® 4500 liquid chromatography mass spectrometry system to actively respond to national and societal appeals, partnering with the state to build multi-layered safety protections and full-process monitoring systems spanning from water sources to faucets. The method described for quantifying 8 nitrosamines offers a simple and rapid solution for the accurate determination of nitrosamine disinfection byproducts in drinking water.

Nitrosamines Analysis Features

- Simple sample pretreatment with Agela Cleanert SPE
- Total sample runtime is less than 10 min, while still achieving separation of nitrosamine analytes on the Phenomenex Kinetex F5 stationary phase
- Sensitive detection limits below 10 ng/L, far below the standard WHO limit of 100 ng/L
- Method can be easily deployed to any SCIEX QTRAP® or triple quadrupole systems.

Experimental

The SCIEX ExionLC™ system and the QTRAP® 4500 mass spectrometry system were employed during method development and data collection.

Sample Pretreatment: SPE

Cleanert PEP-2 SPE 200mg/6mL (Agela Technologies) + activated carbon column.
Procedure for SPE as follows:

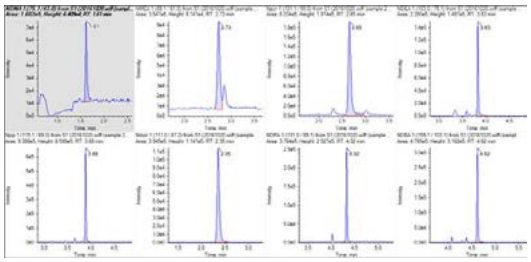

Figure 1: Standard LC-MS/MS Chromatograms of 8 Nitrosamine Compounds. Detection limit below 10 ng/L, far below the standard WHO limit of 100 ng/L.

1. Weigh a defined quantity of ammonium bicarbonate to adjust the water pH to around 8
2. Use dichloromethane and methanol in succession to activate, and use water to equilibrate the SPE column
3. Load the sample at 3-5 mL/min, and rinse with ultrapure water after loading
4. Elute with dicholormethane, evaporate to a defined quantity using nitrogen, and add water to make up to a defined volume for LC-MS/MS analysis to be undertaken
5. Transfer a 0.25 mL aliquot to a polypropylene vial and archive the remaining volume

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Liquid Chromatography Method:

Chromatographic column: Phenomenex 2.6µm F5 100Å. Mobile phase A: water; Mobile phase B: acetonitrile. Gradient elution. Flow rate: 0.5 mL/min. Quantity injected: 20 µL. Time Program details can be found in Table 1.

Table 1: LC Gradient Program. Flow Rate of 0.5 mL/min.

Time (min)	A% (water)	B% (acetonitrile)
0	*95*	*5*
1.5	*95*	*5*
3.5	*10*	*90*
5.1	*95*	*5*
7	*95*	*5*

Mass Spectrometry Method:

Ion source: APCI source, positive ion mode. Ion source parameters: CUR: 30psi GS1: 50psi, NC: 5, TEM: 400°C, CAD: medium. Figure 2 shows the structure of nitrosamines, while Figure 3 shows the parameters of the MRM transitions for each.

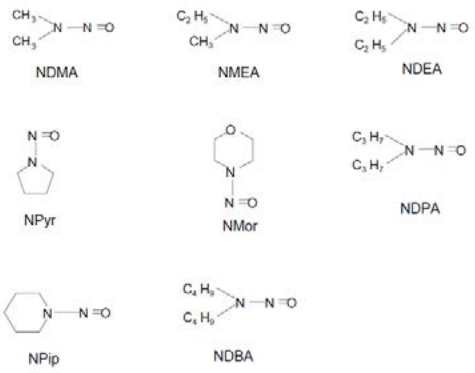

Figure 2: Structures of 8 Nitrosamine Compounds Selected for Quantitative Analysis.

Results

The analyzed standards showed sensitivity down to 10ng/L (Figure 1) and the linear response of all eight nitrosamines ws demonstrated with calibration curve r – values > 0.99 (Figure 4).

	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID	DP (volts)	CE (volts)
1	75.100	43.000	20.0	NDMA 1	40.000	20.000
2	75.100	58.000	20.0	NDMA 2	40.000	16.000
3	89.100	61.000	20.0	NMEA 1	40.000	15.000
4	101.100	55.000	20.0	Npyr 1	40.000	21.000
5	103.000	75.100	20.0	NDEA 1	40.000	15.000
6	115.100	69.000	20.0	Npip 1	40.000	21.000
7	117.000	87.200	20.0	Nmor 1	40.000	16.000
8	131.000	89.100	20.0	NDPA 1	40.000	15.000
9	145.100	97.100	20.0	NDPA-d14	40.000	15.000
10	159.100	103.100	20.0	NDBA 1	40.000	14.000
11	81.000	46.000	20.0	NDMA-d6	40.000	16.000
12						

Figure 3: Eight Nitrosamine Compounds - Mass Spectrometry Parameters. MRM transitions and optimized voltages utilized for the target nitrosamines.

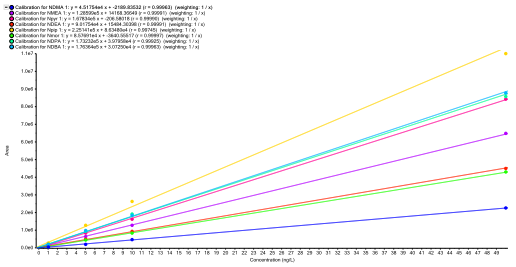

Figure 4: Linear Calibration Curves of 8 Nitrosamine Compounds in Water, Showing Good Linearity (r>0.99).

Conclusions

This paper establishes a comprehensive method for the quantification of nitrosamine compounds, including sample preparation, data acquisition, and data processing using the 4500 QTRAP® system. This method offers clients a comprehensive solution that can be performed on SCIEX 3500, 4500, 5500, and 6500 systems, saving on method development time. The advantages of this method include simple sample pretreatment, brief analysis time, and high sensitivity, allowing accurate and quantitative analysis of nitrosamine disinfection byproducts in drinking water.

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Contents

Analysis of PFAS in drinking water with EPA Method 537.1 and the SCIEX QTRAP® 4500 System

Achieving 537.1 method requirements in a robust 10-minute method

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In the United States, EPA method 537.1 describes the sample preparation, reporting guidelines, and quality control for the analysis of a suite of 14 per- and polyfluorinated substances (PFAS) in drinking water. The EPA 537.1 method guidelines provide some flexibility in the liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Within these guidelines, optimization of certain aspects of the method, such as column chemistry, chromatography, mobile phases, gradient profile, and MS/MS transitions, was performed. Sample preservation and preparation guidelines published in EPA 537.1 are prescriptive and were therefore closely followed.

Table 1. Names, abbreviations, and method detection limits (MDLs) for 14 PFAS compounds included in EPA Method 537.1 along with the reporting limits (MRLs) published by the UCMR3 guidelines 6 of the PFAS compounds. MDLs and MRLs shown as ng/L (ppt) here.

Compound	Abbreviation	Method Detection Limit (ng/L)	UCMR3 Reporting Limit (ng/L)
Perfluorohexane carboxylate	*PFHxA*	***0.09***	**-**
Perfluoroheptane carboxylate	*PFHpA*	***0.1***	***10***
Perfluorooctane carboxylate	*PFOA*	***0.1***	***20***
Perfluorononane carboxylate	*PFNA*	***0.09***	***20***
Perfluorodecane carboxylate	*PFDA*	***0.1***	**-**
Perfluoroundecane carboxylate	*PFUnDA*	***0.1***	**-**
Perfluorododecane carboxylate	*PFDoA*	***0.1***	**-**
Perfluorotridecane carboxylate	*PFTrDA*	***0.2***	**-**
Perfluorotetradecane carboxylate	*PFTeDA*	***0.2***	**-**
Perfluorobutane sulfonate	*PFBS*	***0.1***	***90***
Perfluorohexane sulfonate	*PFHxS*	***0.08***	***30***
Perfluorooctane sulfonate	*PFOS*	***0.1***	***40***
n-ethyl perfluorooctane sulfonamidoacetic acid	*n-EtFOSAA*	***0.1***	**-**
n-methyl perfluorooctane sulfonamidoacetic acid	*n-MeFOSAA*	***0.09***	**-**

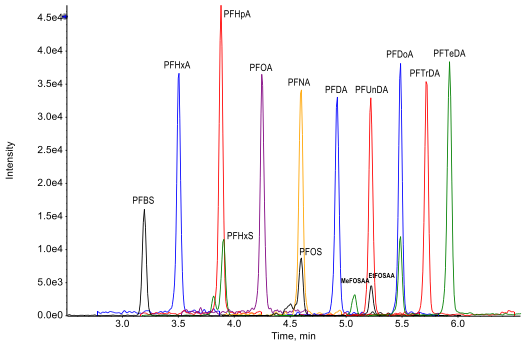

Figure 1. PFAS standard mix at 50 ng/L (ppt) containing all 14 compounds in EPA 537.1 eluting within 6 minutes for an 11 minute total run time with a 10 uL injection.

Key Features of PFAS Analysis on the SCIEX QTRAP® 4500 System

- Robust and reproducible results with qualifying accuracy and precision for calculated concentrations, asymmetry factor, and linearity
- Total sample runtime takes only 8-10 min, depending on autosampler settings and system dead volume
- Sensitive MDLs of 0.08-0.2 ng/L for the entire suite of 14 PFAS compounds

Contents

Sample Preparation

Sample preservation and preparation were performed according to the guidelines in EPA Method 537.1. Briefly, 1 g of Trizma was added to 250 mL polypropylene bottles. Bottles were pre-weighed to calculate the mass of sample collected. Surrogate standards were added to the sample container to achieve a final concentration of 2 ng/L in the 250 mL water sample.

The water samples were extracted using the following procedure with Phenomenex Strata-XL solid phase extraction cartridges (6 mL, 500 mg):

1) Condition SPE tubes with 15 mL of water followed by 18 mL of methanol
2) Add sample to tubes at a flow rate of approximately 10-15 mL per minute.
3) Rinse tubes with 7.5 mL of water and repeat
4) Dry tubes under vacuum for 5 minutes
5) Rinse sample bottle with 4 mL of methanol and transfer methanol to SPE tube while collecting eluent and repeat
6) Evaporate sample to dryness under nitrogen at 40-60°C
7) Reconstitute sample in 1 mL of 96% methanol 4% water containing 1 ng/L of internal standards
8) Transfer a 0.25 mL aliquot to a polypropylene vial and archive the remaining volume

Liquid Chromatography

An Agilent 1200 binary pump was modified by replacing all clear fluoroethylene polymer (FEP) tubing with 1/8 in or 1/16 inch PEEK tubing. A delay column was inserted between the gradient mixing chamber and the autosampler valve to retain contaminants from the eluents or pumps for an extra 1-2 min compared with target analytes eluting from the analytical column.

Table 2: Delay column and analytical HPLC columns from Phenomenex

Column	Name	Dimensions
Delay column	*Phenomenex Luna C18(2)*	*5 µm; 30 x 2 mm*
Analytical column	*Phenomenex Gemini C18*	*3 µm; 50 x 2 mm*

An Agilent 1200 autosampler injected 10 ul of each sample onto the analytical column (Phenomenex Gemini C18), which was heated to 40°C. Gradient separation was performed at a flow rate of 0.6 mL/min using the gradient shown in Table 3.

Table 2: Gradient program

Time (min)	A% (20 mM ammonium acetate)	B% (methanol)
0	*95*	*5*
0.1	*45*	*55*
4.5	*1*	*99*
8	*1*	*99*
8.5	*95*	*5*

Mass Spectrometry

Samples were ionized using negative mode electrospray at the source conditions shown in Table 3 and the Q1/Q3 masses, declustering potentials, and collision energies shown in Appendix Table 1.

Table 2: Source gas, temperature, and voltage settings

Parameter	Value
CAD	*9*
CUR	*30*
GS1	*40*
GS2	*60*
IS Voltage	*-4500*
TEM	*450*

Calibration was performed using an 8-point curve at concentrations of 50, 100, 200, 500, 1000, 2000, 5000, and 10000 ng/L and the concentrations of surrogates and internal standards was 1,000 ng/L in all final sample extracts, standards, method blanks, and quality control samples. Quantitation was performed using MultiQuant™ 3.0.2 using 1.0 point Gaussian smoothing and 1/x weighted linear regression forced through the origin (as required by EPA 537.1). A concentration factor of 250 was applied to samples as a result of the concentration of 250 mL to the final 1 mL extract.

Results

The initial calibration achieved the following guidelines prescribed in EPA method 537.1:

1) Linearity (r^2>0.99) (as shown in Figure 2)
2) Accuracy (+/- 30% for each calibrator)
3) Precision (RSD <20% of 4 replicates of a fortified blank)
4) Asymmetry factor (>0.8 and <1.5 for the first 2 peaks in the chromatogram as shown in Figure 3)
5) Surrogate recovery +/- 30% of expected response
6) Laboratory reagent blanks (LFBs) and field reagent blanks (FRBs) quantitated at <1/3 of the MRL.

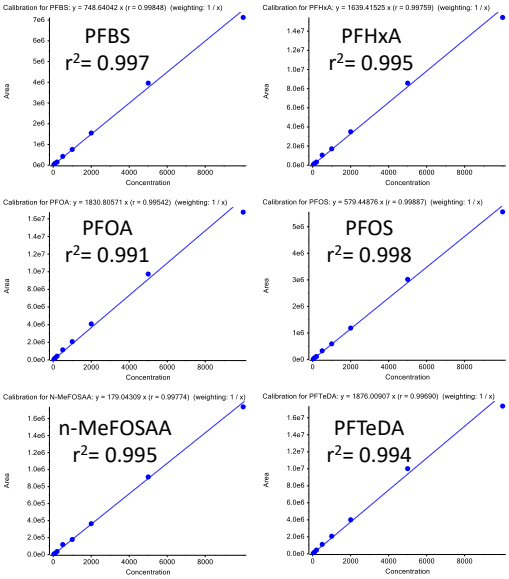

Figure 3: Linearity of 6 of the PFASs analyzed out of the suite of 14 showing r^2>0.99 with a linear fit forced through the origin and 1/x concentration weighting. The other 8 PFAS compounds also showed r^2>0.99.

To calculate method detection limits, 9 water samples were spiked with approximately 0.2 ng/L of each of the 14 PFAS compounds and analyzed following the full analytical protocol. The calculated MDLs shown Figure 1 were calculated according to EPA 537.1 using the mean and standard deviation of the replicated spiked samples. The MDLs for all 14 compounds was below 0.2 ng/L, which highlights the excellent sensitivity of the method.

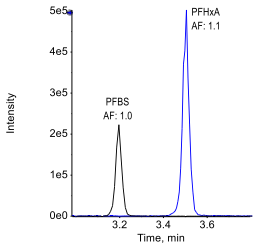

Figure 3: Asymmetry factor (AF) calculated for the first 2 eluting peaks, PFBS and PFHxA, at a mid-point standard concentration of 500 ng/L

After 8 days of analyzing samples to calculate the MDL and other samples, the continuing calibrations still met the requirements of +/- 30% of expected calculated concentration for all 14 analytes, as shown in Table 4.

Table 4: Accuracy of a 50 ng/L calibration standard injected immediately after the initial calibration curve and 6 and 8 days after the calibration curve.

	Days after Calibration					
	0	6	8	0	6	8
Compound	*Calc. Conc. (ng/L)*			*Accuracy (%)*		
PFBS	*44.8*	*54.6*	*57.7*	*90%*	*109%*	*115%*
PFHxA	*54.8*	*52.2*	*52.6*	*110%*	*104%*	*105%*
PFHpA	*55.1*	*50.4*	*58.0*	*110%*	*101%*	*116%*
PFHxS	*47.1*	*53.9*	*49.3*	*94%*	*108%*	*99%*
PFOA	*52.2*	*58.9*	*53.7*	*104%*	*118%*	*107%*
PFNA	*55.1*	*50.9*	*51.3*	*110%*	*102%*	*103%*
PFOS	*47.0*	*45.5*	*48.7*	*94%*	*91%*	*97%*
PFDA	*52.7*	*53.5*	*52.3*	*105%*	*107%*	*105%*
PFUdA	*52.9*	*48.5*	*52.9*	*106%*	*97%*	*106%*
PFDoA	*54.5*	*56.9*	*55.0*	*109%*	*114%*	*110%*
PFTrDA	*52.0*	*54.8*	*51.5*	*104%*	*110%*	*103%*
PFTeDA	*51.6*	*51.0*	*53.0*	*103%*	*102%*	*106%*
n-EtFOSAA	*55.7*	*53.9*	*57.2*	*111%*	*108%*	*114%*
n-MeFOSAA	*56.7*	*61.6*	*58.2*	*113%*	*123%*	*116%*

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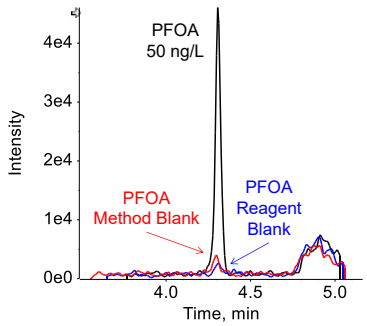

Figure 5: Blank chromatograms showing extremely low contamination in blank samples compared with the 50 ng/L calibration standard. The small peak at 4.9 min is the PFOA contamination eluting from the delay column, which originates from the HPLC pumps and eluent.

Blank samples showed very low responses and were always below the requirement of <1/3 of the MRL. Figure 5 shows the response of a method blank (red), which was a 250 mL water sample taken through the entire sample preparation protocol, and a reagent blank, which was 96% methanol and 4% water prepared in an autosampler vial. A small peak at 4.9 min in the chromatogram in Figure 5 shows presence of PFOA contamination presumably in the HPLC pumps or eluents and demonstrates the adequate separation from the quantitated analyte peak as a result of the delay column installment.

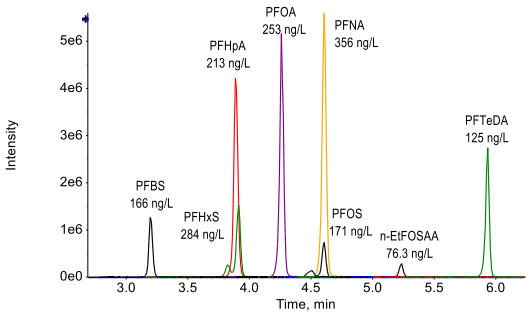

Figure 6: Proficiency testing samples were run to establish method performance met EPA requirements.

PT samples were obtained from ERA and were analyzed along with the MDL replicates. The PT samples were diluted 10:1 with water and analyzed according to the procedures described in this note. The results of the 8 analytes present in PT CAT: 960 were all within +/- 18% of the assigned concentration of the PT study.

The HPLC method and MS/MS analysis for this validated 537 method were both fully compatible with an extended list of 25 analytes. However, the EPA method 537 sample preparation protocol relies on reverse phase, hydrophobic interactions for solid phase extraction (SPE) retention and, therefore, fails to adequately retain shorter chain PFCAs, including perfluorobutane carboxylate and perfluoropentane carboxylate. Modifying this sample preparation method by replacing Strata-XL with Strata-XL-AW (weak anion exchange) solid phase extraction tubes and altering the solvents used during extraction allows a longer, extended list to be analyzed using the same method.

Conclusions

Robust and reproducible results with qualifying accuracy and precision for calculated concentrations, asymmetry factor, and linearity were achieved in a single ten-minute LC-MS/MS acquisition on the SCIEX QTRAP® 4500 system. Following stringent protocols for sample preparation and preservation defined by the EPA 537.1 method, method optimization steps were taken to continue to improve PFAS analysis workflow and quantitative performance. Background contamination was minimized through LC system adjustments (replacing FEP tubing and installing delay column). Analysis of shorter-chain PFCAs was improved through selection of weak anion exchange sorbent for sample SPE. Sensitive MDLs of 0.08-0.2 ng/L for the entire suite of 14 PFAS compounds were achieved, all of which meet or exceed the requirements of the US EPA's UCMR3 list for drinking water, and method robustness was demonstrated by sustained accuracy of measured concentration in a QC sample over eight days without need for re-injection of calibration standards.

Contents

Appendix Table 1. MRM transitions, instrument voltage parameters, and retention times.

Name	Q1	Q3	RT (min)	DP	CE
PFBS	298.9	80	3.2	-20	-56
PFBS_2	298.9	99	3.2	-20	-46
13C3_PFBS	302	80	3.2	-20	-56
PFHxA	313	269	3.5	-10	-14
PFHxA_2	313	119	3.5	-10	-25
13C2_PFHxA	315	270	3.5	-10	-14
13C5_PFHxA	318	273	3.5	-10	-14
PFHpA	363	319	3.9	-10	-14
PFHpA_2	363	169	3.9	-10	-25
13C4_PFHpA	367	322	3.9	-10	-14
PFHxS	399	80	3.9	-20	-74
PFHxS_2	399	99	3.9	-20	-60
13C3_PFHxS	402	80	3.9	-20	-74
PFOA	413	369	4.3	-10	-14
PFOA_2	413	169	4.3	-10	-26
13C2_PFOA	415	370	4.3	-10	-14
13C8_PFOA	421	376	4.3	-10	-14
PFNA	463	419	4.7	-10	-16
PFNA_2	463	169	4.7	-10	-26
13C9_PFNA	472	427	4.7	-10	-16
PFOS	499	80	4.7	-20	-95
PFOS_2	499	99	4.7	-20	-87
13C4_PFOS	503	80	4.7	-20	-95
13C8_PFOS	507	80	4.7	-20	-95
PFDA	513	469	5	-10	-17
PFDA_2	513	169	5	-10	-27
13C2_PFDA	515	470	5	-10	-17
13C6_PFDA	519	474	5	-10	-16
PFUdA	563	519	5.3	-10	-18
PFUdA_2	563	169	5.3	-10	-28
13C7_PFUdA	570	525	5.3	-10	-18
N-MeFOSAA	570	419	5.2	-50	-28
N-MeFOSAA_2	570	483	5.2	-50	-22
d3-MeFOSAA	573	419	5.2	-50	-28
N-EtFOSAA	584	419	5.3	-50	-28
N-EtFOSAA_2	584	526	5.3	-50	-28
d5-EtFOSAA	589	419	5.3	-50	-28
PFDoA	613	569	5.6	-10	-18

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Improving Identification and Quantification of Polar Herbicides by CESI-MS

Achieving better differentiation of glyphosate, fosetyl aluminum and their degradation products

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Overview

Who Should Read This: Senior Scientists, Lab Directors

Focus: Advantages of CESI-MS for separating, identifying and quantifying the polar herbicides glyphosate and fosetyl aluminum, and their degradation products.

Goals: Develop an effective CESI-MS method for separating, identifying and quantifying polar herbicides and compare the selectivity, accuracy and reproducibility of that method to those of an approved, currently-used LC-MS method.

Problem: Concerns about the safety of glyphosate-based herbicides (GBHs) have made it essential to be able to detect glyphosate in foods (especially fruits and nuts) and distinguish it from other alternative herbicides such as fosetyl aluminum. Current LC-MS methods have significant limitations, including ion suppression, retention time instability and problems in distinguishing between degradation products of these herbicides (Figure 1). Both glyphosate and fosetyl aluminum are regulated but false positive identification (ID) and inaccurate quantitation of their degradation products, phosphate and phosphonate, is possible using current LC-MS methods. Therefore, a method is needed that provides accurate ID and quantitation of these degradation products.

Results: The developed CESI-MS method demonstrated an excellent ability to distinguish between glyphosate and its degradants, and between similar degradation products of another widely-used herbicide, fosetyl aluminum. It also demonstrated better migration/retention time stability and quantitative linearity than the LC-MS method.

Key Challenges:

- Separation of highly polar molecules by LC requires either time-consuming analyte derivatization prior to reverse-phase LC, or reliance on less reliable LC techniques, e.g. HILIC or anion exchange chromatography
- LC-MS methods suffer from a variety of issues, including: derivatization selectivity, ion suppression due to matrix effects, and retention time instability

- LC-MS methods frequently have difficulty resolving phosphate and phosphonate, the final degradation products of glyphosate and fosetyl aluminum, respectively

Key Features:

- Capillary electrophoresis is well suited to the separation of polar herbicides
- The CESI-MS method provided excellent specificity, easily resolving and identifying glyphosate, fosetyl aluminum, and many of their degradation products
- The CESI-MS method demonstrated very good migration time stability over more than 160 runs
- The CESI-MS exhibited excellent quantitative linearity when analyzing phosphonate, the degradation product of fosetyl aluminum, in matrices

Differentiating Glyphosate, Other Herbicides and Their Degradation Products

Glyphosate is a common, broad-spectrum, systemic herbicide widely used to kill weeds that compete with crops. Concerns about the safety of glyphosate[1,2] have led to increasing restrictions on glyphosate-based herbicides (GBHs), most recently in the European Union.[3] As such, it is increasingly important to develop robust analytical methods with the sensitivity and selectivity to identify and quantify glyphosate and its degradation products in foods and differentiate them from other herbicides such as fosetyl aluminum.

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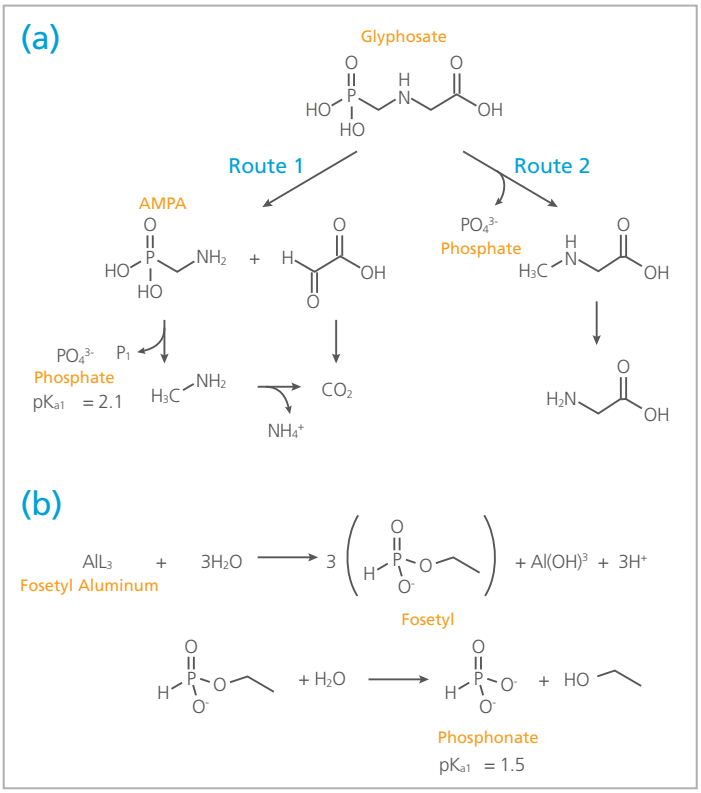

Figure 1: Degradation pathways for (a) glyphosate and (b) fosetyl aluminum.

Limitations of LC-MS Methods

Several LC-MS -based methods are currently used to analyze glyphosate and its degradation products, with many listed in the Quick Polar Pesticides (QuPPe) Method document created by the EU Reference Laboratories for Residues of Pesticides.[4] The methods use HILIC, porous-graphitized carbon columns or anion exchange chromatography coupled with mass spectrometry. Reverse-phase LC methods have also been used but require analyte derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) before sample analysis.[5] While LC-MS methods generally can differentiate glyphosate and fosetyl aluminum (Figure 1), these methods can suffer from derivatization selectivity, matrix effects, ion suppression, and poor retention time reproducibility. Additionally, LC-MS methods are generally not suitable for resolving phosphate and phosphonate, the final degradation products of glyphosate and fosetyl aluminum, especially in real-world matrices.[4]

Advantages of CESI-MS

Capillary electrophoresis (CE) is well-suited to the analysis of polar ions and has already proven useful in the analysis of pesticides.[6] The mechanism of separation is by differences in pKa and hydrodynamic radii. In the case of phosphate and phosphonate, they differ in pKa by 0.6 units (Figure 1). Integration of capillary electrophoresis and electrospray ionization (CESI) into a single dynamic process facilitates the mass spectrometric use of CE detection and analysis. The developed CESI-MS method readily differentiated glyphosate, fosetyl aluminum and their degradation products (Figure 2). It demonstrated far better migration/retention time stability than a corresponding LC-MS method (Figure 3). Finally, quantitative CESI-MS/MS analysis of phosphonate in real-world nut extracts showed exceptional linearity while the corresponding anion-exchange LC-MS/MS method exhibited significant ion suppression due to matrix effects (Figure 4).

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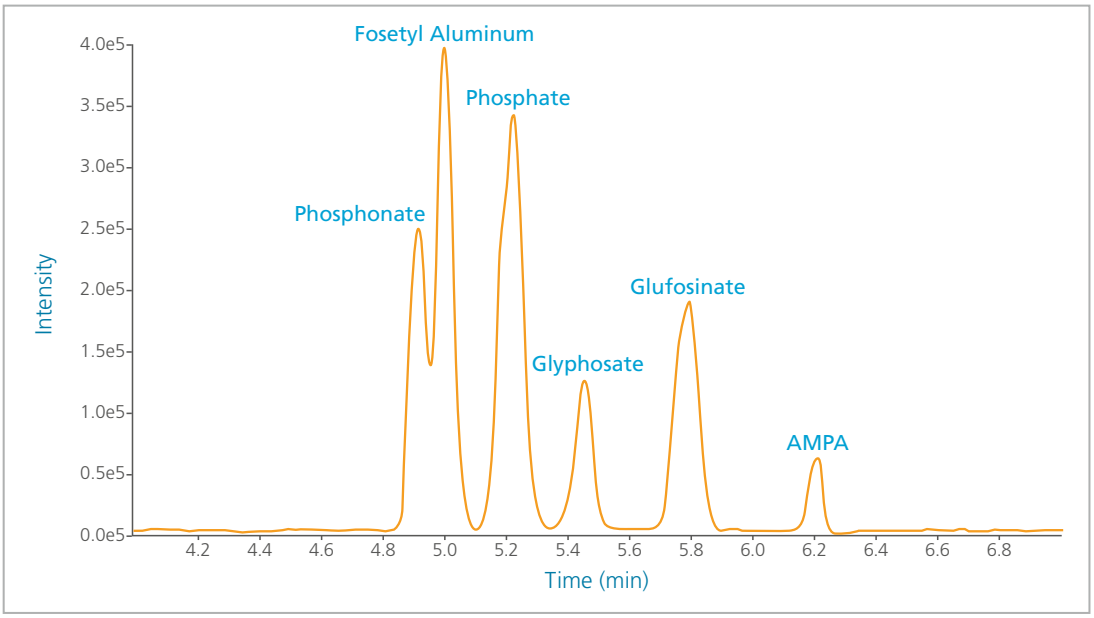

Figure 2: CESI-MS provides clear separation and detection of glyphosate and three of its degradation products: glufosinate, AMPA and phosphate, along with fosetyl aluminum and one of its degradation products, phosphonate.

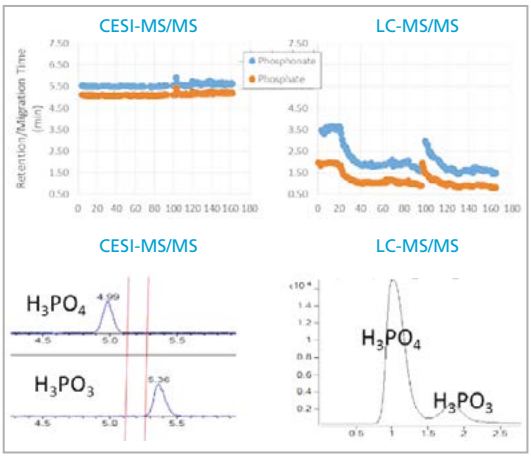

Figure 3: Migration/retention times for phosphonate and phosphate across more than 160 analyses, with sample electropherograms (CESI-MS/MS) and chromatograms (LC-MS/MS). CESI-MS proved far more stable over time and baseline separation was achieved only in the CESI-MS analysis.

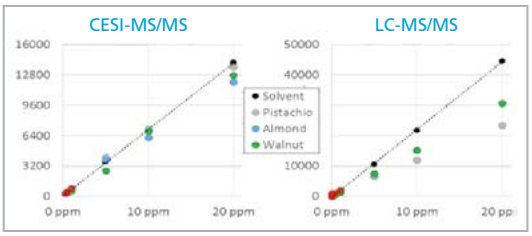

Figure 4: CESI-MS/MS and anion-exchange LC-MS/MS quantification of phosphonate spiked into 0.1% formic acid (solvent) and nut extracts. Significant ion suppression due to matrix effects is clearly visible in the LC-MS/MS results, but not in the CESI-MS/MS results. Fit lines are for the solvent curves only.

Contents

References

1. Myers, J.P. et al., Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement, *Environmental Health*, 2016 15:19.
2. World Health Organization's International Agency for Research on Cancers Monographs on the Evaluation of Carcinogenic Risks to Humans, *Glyphosate*, 2015, Volume 112.
3. http://europa.eu/rapid/press-release_MEMO-16-2012_en.htm
4. Anastassiades, M. et al., Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction, with Methanol (QuPPe-Method), *EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM)*, 2016, Version 9.1. http://www.eurl-pesticides.eu/.
5. Schreiber, A and Cabrices, O.G., Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides, 2013, AB SCIEX Publication number: 8013813-01.
6. El Rassi, Z., Capillary Electrophoresis of Pesticides, *Electrophoresis*, 1997, 18, 2465 – 2481.

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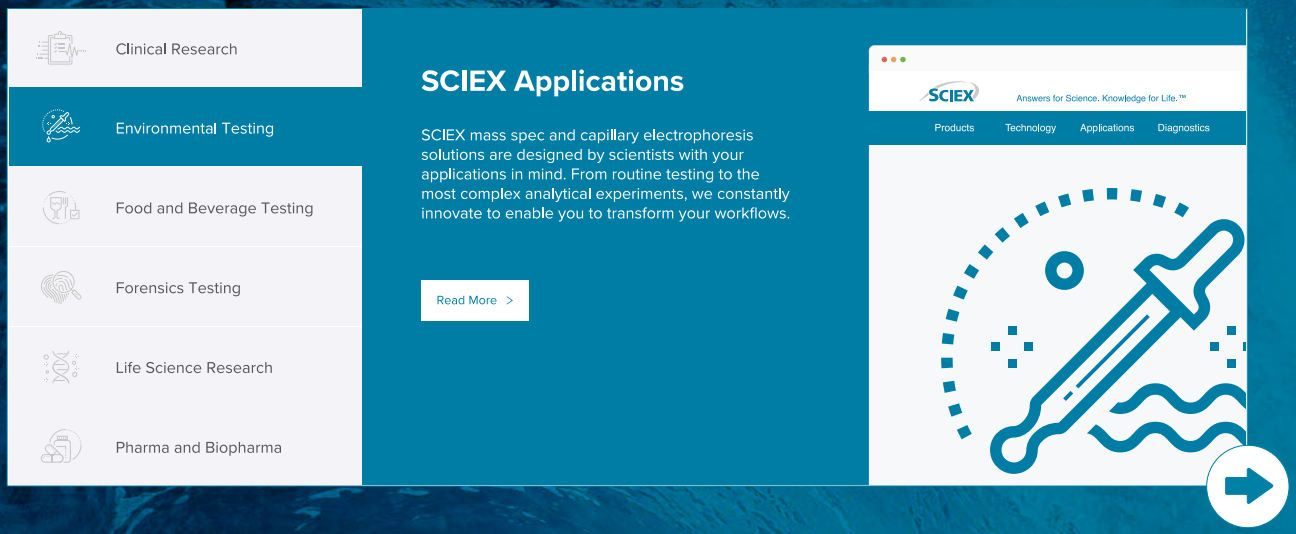

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