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Analytical solutions:

Meeting the requirements of US and European water standards

ThermoFisher SCIENTIFIC

Foreward

The quality of the water in our rivers, lakes and seas is of significant concern for both the organisms that inhabit these environments and those that rely on it as a water source – ourselves included. Around 99% of the world's drinking water comes from a combination of groundwater, rivers and lakes (including man-made reservoirs), with the remaining 1% produced by desalinating sea water – an essential process for countries where access to other water supplies is limited.

Effectively monitoring and regulating water sources is therefore of vital importance. Ensuring that water supplies are maintained at the necessary purity to sustain aquatic life and that they can be sustainably extracted and efficiently treated for drinking is a continuous challenge. To address this, global governments and regulatory bodies have established extensive legislation based on the identification, quantification and regulation of known pollutants, such as pesticides, pharmaceuticals, anions and trace metals. Additionally, they provide a framework for addressing emerging contaminants. The most well-known of these regulations are those developed by the United States Environmental Protection Agency (US EPA) and the European Union (EU), the latter in the form of the Water Framework Directive (EU WFD). Between them, these regulations provide a foundation for water quality control legislation around the world.

This eBook covers the scope and detail of the EPA and EU WFD regulations for both known and emerging contaminants in water sources. It also provides details about the instrumentation developed by Thermo Fisher Scientific to enable laboratories to meet the analytical requirements of these regulations.

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EPA regulations and water safety: meeting the challenges

Established in 1970, the United States Environmental Protection Agency (US EPA) is an independent agency of the United States federal government that works to protect human health and the environment. Amongst its many responsibilities the EPA is responsible for ensuring that water is safe from harmful contaminants. Here Richard Jack, Senior Director, Vertical Marketing from Thermo Fisher Scientific takes us through some of the EPA's safety standards and how labs meet these, today and into the future.

Q: What safety standards does the US Environmental Protection Agency (EPA) have in place to ensure our water is safe from hazardous contaminants?

Richard Jack (RJ): The United States enjoys one of the world's most reliable and safest supplies of drinking water. Congress passed the Safe Drinking Water Act (SDWA) in 1974 to protect public health, by regulating public water systems. The Safe Drinking Water Act (SDWA) requires EPA to establish and enforce standards that public drinking water systems must follow. EPA delegates primary enforcement responsibility (also called primacy) for public water systems to states and Indian Tribes if they meet certain requirements. Approximately 150,000 public water systems provide drinking water to most Americans.

There are specific regulations for organic and inorganic contaminants, some key contaminants such as arsenic, lead and copper rules and other toxic metals. Organic / chemical contaminants capture broader topics due to the wide variety of pesticides, polychlorinated biphenyls (PCBs), toxic ions and disinfection by-products (DBPs). These DBPs are unique for drinking water, are highly toxic, and therefore have specific regulations requiring enforcement.

In the US, drinking water is federally regulated, meaning that each city must meet the same regulatory requirements. The states can develop lower regulatory limits but must first meet the basic minimum requirements set forth in the safe drinking water act. An example of where the states have made additional regulatory adjustments are for the toxic ion, perchlorate, a by-product of fertilizers and rocket fuels which have affected ground waters in certain parts of the US. Ground waters are important sources for drinking water in the US.

Q: What components of wastewater and other environmental samples are labs required to analyze?

RJ: The basic components in wastewaters are general analysis methods such as total organic carbon (TOC), total nitrogen (TN), and total phosphorous (TP). Additional parameters include pH, conductivity and biochemical and chemical oxygen demand. These broad spectra of analyses provide general indications of water health. However, more detailed analysis is also required for metals, anions, pesticides, pharmaceuticals, PCBs. Typically, the specific inorganic and organic contaminants depend on a discharge permit for a particular wastewater plant. Because our knowledge of toxicity, persistence and contaminant

scope is becoming more well known, updates to these regulations are taking place in certain countries.

Waste or solid waste is regulated and tested separately from water and air analysis. The chemicals are often similar and are regulated in terms of disposal and containment. It is important to note that the definition of solid waste is not limited to wastes that are physically solid. Many solid wastes are liquid, semi-solid, or contained gaseous material. For example, solid waste leachates impact groundwaters and heavy metals impact soils destined for agricultural purposes.

The US EPA regulates solid waste through the Resource Conservation and Recovery Act (RCRA), established in 1976. Within the regulations there are parameters that distinguish solid waste from hazardous waste. The RCRA defines "solid waste" as garbage or refuse, sludge from a wastewater treatment plant, water supply treatment plant, or air pollution control facility and other discarded material, resulting from industrial, commercial, mining, and agricultural operations.

Q: What are some of the biggest challenges faced by environmental testing labs? And how do they overcome these challenges?

RJ: An environmental lab faces many challenges that pertain to:

1. lab operations, and sample tracking
2. quality control
3. maintaining regulatory compliance
4. maintaining instruments to keep them up and running
5. training of personnel
6. service and support

As samples are split and sent to different labs, sample tracking through a lab and the instrumentation must be very accurate. Lab Information Management Systems (LIMS) are [commonly] used to accurate track samples from collection to report generation. Sample analysis and accuracy needs to be maintained through a robust Quality Assurance and Quality Control (QA/QC) program. This requires more standards, spikes samples, and recoveries to be evaluated to ensure sample measurements are accurate.

Probably the biggest constraint for laboratories is instrument uptime. If instruments aren't running, samples aren't being analysed and revenue cannot be generated. Robustness in instrumentation, including the hardware and software, is critical for any lab. To maintain robust analysis, labs require service and support within sort time frames (48 hrs). Typically, labs have inhouse personnel that can perform basic instrument maintenance. Additionally, they can purchase regular maintenance from vendors in order to maintain the required limits of detection (LOD) and limits of quantification (LOQ). Today's analytical instruments are extremely sensitive, so they usually meet LOD, LOQ requirements.

Depending upon the sample type being measured there maybe additional considerations. These include, have interfering matrices, such as salts, which can precipitate and clog an instrument, organic matter that can build up and clog analytical columns, and in some cases heavy metals, which can precipitate in a flow path. It is worth noting that sample preparation is a key component of soil analysis since extraction of a contaminant from soil is much more complicated and has considerably more matrix interferences that affect analysis accuracy. Thus, sample preparation is a major cost challenge as more labor is required and more sample prep products such as filters and techniques require expensive solvents and manual labor. Wastewaters, ground waters and surface waters are also considered dirty matrices and extra procedures are often needed to remove matrix.

An example of sample preparation for dirty waters is solid phase extraction (SPE). SPE columns are designed to capture the contaminant of interest and provide a wash step where the interference is removed. The contaminant is then eluted and concentrated before injection into the analytical instrument. To help minimize labor costs, automated sample preparation is also available, such as Accelerated Solvent Extraction (ASE®) for solids and automated extraction of water devices such as AutoTrace®.

Q: How do labs safeguard themselves from updates to EPA regulations?

RJ: The EPA informs laboratories through public announcements to certified laboratories. For example, the EPA Office of Water issues Method Update Rules which typically cover a broad range of methods and updates for new technology, calibration, compliance etc. Often these updates provide opportunities for the use of new instruments, new column formats, modified QA/QC requirements, detectors, sample preparation step modifications as well as many other cost saving attributes. As an example, there are several EPA Departments validating methods for per-, and polyfluorinated alkyl substances (PFAS) which use LSMS detection. Some include solid phase extraction for sample preparation

while other do not. New methods are being validated for drinking-, surface-, waste-, and ground waters as well as soils and sediments.

Labs must also maintain ongoing certifications not related to updates in regulations to be in regulatory compliance. Monitoring is one of the key components EPA uses to ensure that the regulated community obeys environmental laws and regulations. Compliance monitoring includes:

- Formulation and implementation of compliance monitoring strategies
- On-site compliance monitoring: compliance inspections, evaluations, and investigations (including review of permits, data, and other documentation)
- Off-site compliance monitoring: data collection, review, reporting, program coordination, oversight, and support

EPA inspectors and auditors, as well as 3rd party contract auditors, assess a laboratory's proficiency through a variety of factors. In this way, the EPA works with labs to ensure they can meet necessary analysis and reporting standards. Lab audits are important because they ensure that correct methods are used, that methods are applied correctly, and that results meet needs and expectations.

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WHITE PAPER 72958

Fast determination of haloacetic acids in drinking water

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Automation, eluent generation, haloacetic acids (HAA), ion chromatography (IC), IC-MS/MS, mass spectrometry (MS)

Introduction

As a result of current government proposals and developments, haloacetic acids (HAA) are in the focus of modern water analysis. The established methods use gas chromatography with electron capture detection (GC-ECD) or mass spectrometry (GC-MS). However, the drawback of these methods is the need for time-consuming derivatization and multiple extraction steps. Can the analysis be simplified? Can sensitive and rapid detection be achieved without sample pretreatment? In this paper, these questions are answered based on current developments in IC-MS/MS.

Discussion

Right2Water

In response to the "Right2Water" initiative, supported by 1.6 million Europeans, the European Commission proposed a revision of the Drinking Water Directive in January 2018.[1] The obligatory and extended list of criteria contains 18 new or revised entries, including chlorate and HAAs.[2]

Organic molecules present in the feedwater of drinking water production, as well as naturally occurring or anthropogenic bromide and iodide, react with chlorine-containing disinfectants to form halogenated intermediates from which the HAAs originate as by-products.

However, the U.S. Department of Health and Human Services[3], as well as other authors[4-7], evaluated the available scientific data as being insufficient to establish a safe link between human cancer and individual HAA, subclasses and the class of HAAs. Other studies on by-products of water disinfection indicate a potential cancer risk from chlorinated water and underline the relevance of animal cancer studies for humans.[3]

Accordingly, the Annex to the EU proposal lists nine representative HAAs (9HAA) whose total content may not exceed 80 µg/L: monochloro (MCAA), dichloro (DCAA) and trichloroacetic acid (TCAA), mono- (MBAA) and dibromoacetic acid (DBAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), dibromo-chloroacetic acid (DBCAA), and tribromoacetic acid (TBAA).

Analytics

Common gas chromatographic methods are based on liquid-liquid extraction and derivatization of HAAs either with diazomethane[8] or with methanol[9] in conjunction with electron capture detectors or mass spectrometers. These methods verify five of the nine proposed HAAs. They are labor-intensive and time-consuming. Also, diazomethane can only be used in Sweden with the permission of the proper authority because of its listing as a carcinogenic air pollutant in the workplace.[10]

Consequently, there is an increasing interest in simplified analytical methods for the determination of 9HAA. The low pKa values of HAAs[11] suggest the use of anion exchange chromatography.

IC-MS/MS

Currently, only one validated EPA method based on IC describes the separation of 9HAA in drinking water.[12] To minimize unwanted sensitivity losses in MS (ion suppression), the authors describe mandatory requirements for the chromatographic separation: The target components must be separated from the common anions in the drinking water, the samples must be directly injected. Filtration and sample pretreatment by solid phase extraction are not permitted. The effluent of the chromatographic system needs to be low conductive, and the EPA method specifies a value of less than 2.5 µS/cm. The separation is accomplished using a polymeric, high-capacity separation phase (Thermo Scientific™ Dionex™ IonPac™ AS24). Before conductivity and MS-detection, a continuously electrolytically regenerated suppressor (Thermo Scientific™ Dionex™ ASRS™) is used, converting the eluent (KOH) into water and the eluting anions into their corresponding acids, thus improving the sensitivity and selectivity of both detectors. Applying these experimental conditions the trace determination of 9HAAs is facilitated even in the presence of high concentrations of the main anions like 320 mg/L chloride, 250 mg/L sulfate, 150 mg/L bicarbonate, and 20 mg/L nitrate (LSSM)[12]. The cycle time of the described EPA method is 56 min.

Optimizations

The economics of a modern analysis laboratory can be improved by reducing the time required to determine a sample. Faster chromatography can be achieved, for example, by changing the column properties. With this in mind, the Thermo Scientific™ Dionex™ IonPac™ AS31 column was developed. On this column, the separation of the 9HAA with an electrolytically generated hydroxide gradient is achieved in less than 35 min. This results in a time savings of more than 30% (Figure 2).[13] Under these conditions, the main components chloride, sulfate, and bicarbonate/carbonate elute from the Dionex IonPac AS31 column in one peak without impairing the resolution of the HAAs. Figure 2 shows only the conductivity detection. The use of a continuously electrolytically regenerated suppressor is a prerequisite for the continuous desalination of the highly alkaline eluent, allowing connection of the IC to MS detectors. At the same time, the device configuration is clearly defined, and the traceability of the analytical measurement is ensured. Modern ion chromatographs, therefore, have automatic logging of the consumables used in the device, so that configuration information can readily be extracted at any time from the analytical raw data.[14,15] Figure 3 shows the detection of 9HAAs, 2,2-dichloropropionic acid (dalapon), and bromate in the targeted selected ion monitoring mode of the mass spectrometer. The quantities added to the drinking water matrix (LSSM, see Reference 12) were 4 µg/L, each. The determination of such trace levels and below is possible. The grayed segments in the figure represent the retention time windows during which the main components elute, and the chromatographic effluent is diverted to waste via the switching valve shown in Figure 1. This matrix diversion facilitates the trace determination of HAAs by further reducing potential ion suppression effects in the MS.

Various methods for the liquid chromatographic analysis of 9HAAs are found in the literature. Some selected examples describe HILIC MS/MS[16] or IC-MS/MS with KOH/K_2CO_3 eluents in conjunction with discontinuously regenerated packed bed suppressors,[17] RP-MS/MS,[18] or IC-HRMS in conjunction with SPE[19]. In most of these articles, obligatory demands of the validated EPA-method for the determination of 9HAAs are not met.

As long as no validated ISO methods are available for the analysis of 9HAAs with IC/LC, it is suggested to follow the EPA evaluation procedure and prerequisites when testing any newly developed method.[12]

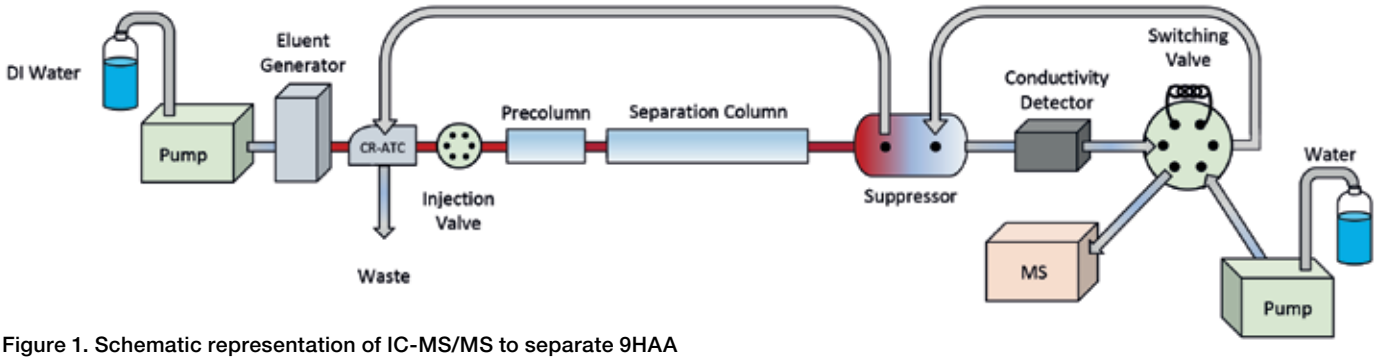
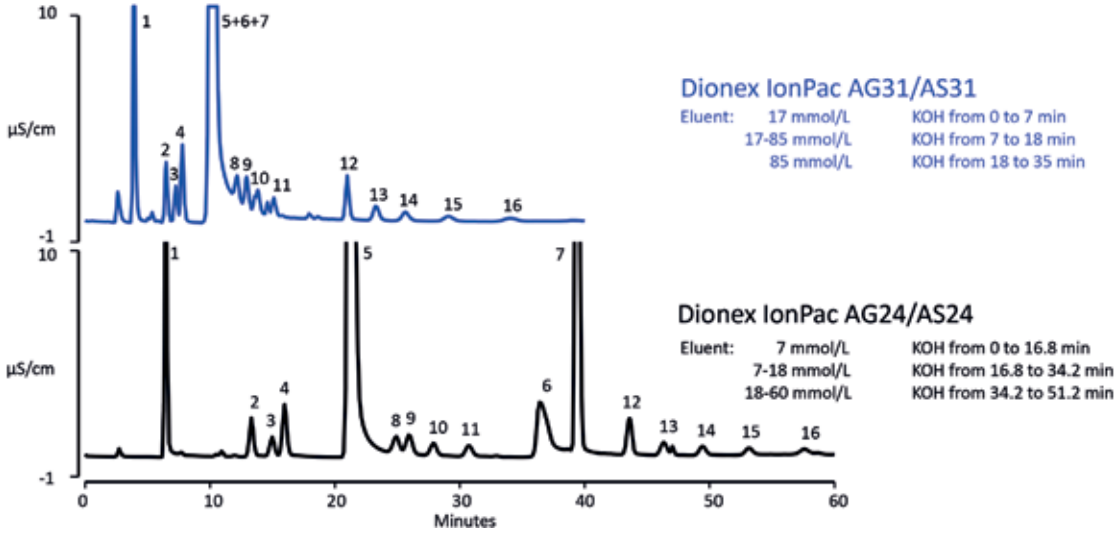


Figure 1. Schematic representation of IC-MS/MS to separate 9HAA



Peak		mg/L
1	Fluoride	NQ
2	MCAA	1.0
3	MBAA	1.0
4	Bromate	1.0
5	Chloride	NQ
6	Sulfate	NQ
7	Carbonate	NQ
8	Dalapon	1.0
9	DCAA	1.0
10	BCAA	1.0
11	DBAA	1.0
12	Nitrat	NQ
13	TCAA	1.0
14	BDCAA	1.0
15	DBAA	1.0
16	TBAA	1.0

Figure 2. Comparison of chromatographic selectivities. Instrument: Thermo Scientific™ Dionex™ ICS-6000. Columns and conditions: see illustration. Detection: Suppressed Conductivity (Thermo Scientific™ Dionex™ ADRS Anion Dynamically Regenerated Suppressor)

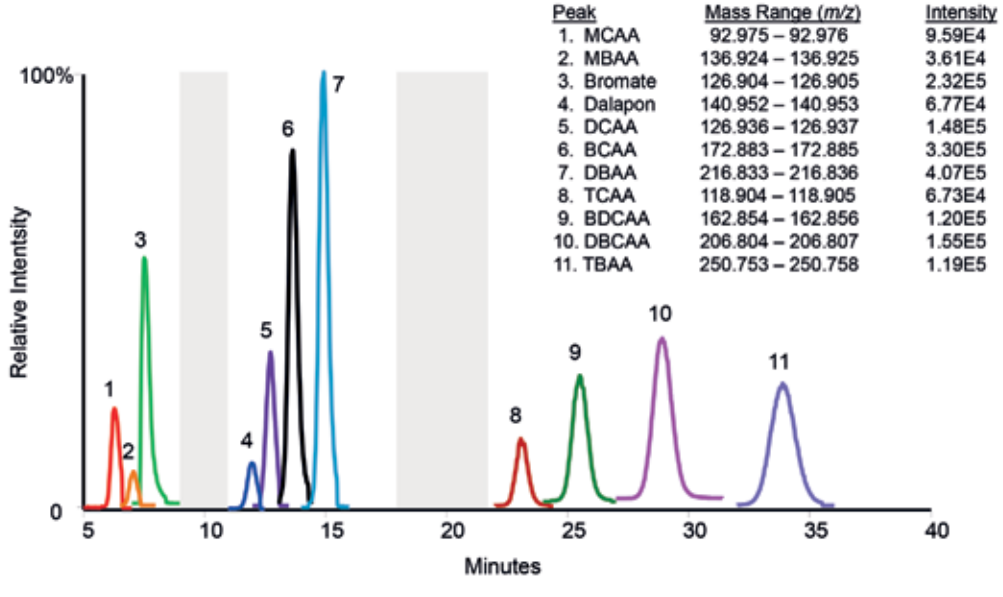


Figure 3. Separation and detection of the 9HAA, bromate and dalapon, 4 µg/L (each) in drinking water (LSSM, s. [12]). Dionex ICS-6000 system with Dionex IonPac AS31 column (15 °C), KOH gradient (Eluent Generator), 0.3 mL/min, 100 µL injection, Conductivity detection (Dionex ADRS). MS detection: Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ mass spectrometer, Targeted SIM XIC.

Summary

IC can be used to directly determine the 9HAAs and bromate, chlorate and dalapone as listed in the EU proposal without sample preparation. Mass spectrometry detection provides the sensitivity and selectivity required to achieve reliable analytical results. In conjunction with "Reagent-Free IC" (RFIC), i.e., IC without manual preparation of eluents or regenerants, a high degree of automation is achieved, which yields high reproducibility of the separations and minimal labor in the laboratory. The use of a new stationary phase (Dionex IonPac AS31) reduces the run times by more than 39% in comparison to EPA Method 557, and all the key requirements for analytical validation as in EPA Method 557 are met. The method described in EPA Method 557 should be used as a reference for the evaluation of new methods.

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APPLICATION NOTE 10522

Optimized GC-MS solution for semivolatiles (SVOC) analysis in environmental samples in compliance with the U.S. EPA Method 8270D

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Keywords

GC-MS, Semivolatiles, Helium Saver, ISQ 7000, ExtractaBrite, EPA 8270, SVOC, SVOA, semivolatile organic compound, BNA, base neutral acids, organic contaminants

Introduction

The United States Environmental Protection Agency (U.S. EPA) released the first Semivolatile Organic Compounds (SVOC) method by Gas Chromatography/Mass Spectrometry (Method 8270) at the end of 1980. It is a common method used in almost all environmental laboratories looking to analyze semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media, and water.[1] Since then, single quadrupole mass spectrometers have become much more sensitive and the source fragmentation has changed. Many original assumptions[2] about the origin and nature of the ion species have proven to be wrong or require correction, while the new generations of the mass spectrometers have proven to provide more response in the high-mass region,[3] resulting in adjustment of the tuning criteria to be met.[4] To adjust to these changes, the EPA has changed the ion abundance criteria for the passing of DFTPP ion ratio criteria in EPA Method 8270D.

This application note shows how the Thermo Scientific™ ISQ™ 7000 single quadrupole GC-MS system can meet Method 8270D requirements with the extended dynamic range detection system. The working method range was shown to be 0.2–200 ppm using the same column.

Particular attention has been posed on maximizing the uptime of the instrument, as required by high-throughput laboratories. The innovative Thermo Scientific™ NeverVent™ technology available on the ISQ 7000 GC-MS system is a unique solution for speeding up the routine maintenance operations, saving the time typically required to vent the MS system and re-establish the vacuum conditions.

The new Thermo Scientific™ Instant Connect Helium Saver Injector was also assessed in this application note to show that significant financial costs savings can be realized throughout the lifetime of a GC-MS instrument without compromising the instrument's performance.

Experimental

The method was tested on five ISQ 7000 GC-MS systems equipped with the Thermo Scientific™ ExtractaBrite™ ion source to assess method transferability and instrument-to-instrument variability. Both ranges (0.2–50 ppm and 2–200 ppm) were validated using the Instant Connect Helium Saver Injector (P/N 19070013) and the Thermo Scientific™ Instant Connect Split-Splitless (SSL) Injector module (P/N 19070010). The column used was a Thermo Scientific™ TraceGOLD™ TG-5MS GC Column with 5 m guard, 30 m × 0.25 mm × 0.25 µm (P/N 26098-1425). A Thermo Scientific™ Injection Port Deactivated Liner 4 mm ID × 105 mm (P/N 453A1925) was selected for the Split-Splitless injection port. The ISQ 7000 GC-MS system operated in full-scan mode and the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software was used to acquire, process, and report data. The operating parameters for the Thermo Scientific™ TRACE™ 1310 GC system are reported in Table 1a (splitless method, range 0.2–50 ppm) and Table 1b (split method, range 2–200 ppm). The ISQ 7000 single quadrupole MS operating conditions are detailed in Tables 2a and 2b.

Table 1a. TRACE 1310 GC system parameters for splitless method.

Injection Volume (µL)	1.0
Liner	Deactivated Splitless Liner
Inlet Temp (°C)	270
Inlet Module and Mode	SSL in Surge Splitless at 345 kPa for 0.6 min
Splitess Time (min)	0.6
Split Flow (mL/min)	50
Oven Temperature Program	
Initial Temperature 1 (°C)	35
Hold Time (min)	2.25
Rate (°C/min)	25
Temperature 2 (°C)	100
Hold Time (min)	0.1
Rate (°C/min)	30
Temperature 3 (°C)	280
Hold Time (min)	0.1
Rate (°C/min)	10
Temperature 4 (°C)	320
Hold Time (min)	5.00

Table 1b. TRACE 1310 GC system parameters for split method.

Injection Volume (µL)	1.0
Liner	Deactivated Splitless Liner
Inlet Temp (°C)	310
Inlet Module and Mode	SSL in Split Mode
Split Ratio	10:1
Split Flow (mL/min)	15
Carrier Gas (mL/min)	He, 1.5
Oven Temperature Program	
Initial Temperature 1 (°C)	35
Hold Time (min)	2.25
Rate (°C/min)	25
Temperature 2 (°C)	100
Hold Time (min)	0.1
Rate (°C/min)	30
Temperature 3 (°C)	280
Hold Time (min)	0.1
Rate (°C/min)	10
Temperature 4 (°C)	320
Hold Time (min)	5.00

Table 2a. ISQ 7000 Single Quadrupole MS parameters for splitless method.

Transfer Line Temp (°C)	300
Ion Source	ExtractaBrite
Ion Source Temp (°C)	300
Ionization Mode	EI
Electron Energy (eV)	70
Acquisition Mode	Full-scan
Scan Range (*m/z*)	35–500
Emission Current (mA)	10
Dwell Time	0.1

Table 2b. ISQ 7000 Single Quadrupole MS parameters for split method.

Transfer Line Temp (°C)	310
Ion Source	ExtractaBrite
Ion Source Temp (°C)	300
Ionization Mode	EI
Electron Energy (eV)	70
Acquisition Mode	Full-scan
Scan Range (*m/z*)	35–500
Emission Current (mA)	15
Dwell Time	0.1

Tuning for DFTPP

The ISQ 7000 MS system was tuned with a built-in EPA 8270D specifically designed tune (DFTPP Tune). This assures fulfillment of all method requirements in terms of ion abundance criteria. A tune verification DFTPP solution was injected to verify that the ISQ 7000 GC-MS system met the tuning requirements shown in Figure 1. Chromeleon CDS software has a dedicated reporting package for environmental laboratories, and automatically reports tune evaluation performance with a Pass/Fail indicator (Table 3).

Standard and sample preparation

Standards (Restek 8270 MegaMix Cat. No. 31850, AccuStandard Internal Standard Cat. No. Z-014J, AccuStandard Surrogate Cat No. M-8270-SS) were prepared in methylene chloride, and the internal standards were spiked at a concentration of 5 ppm for both the splitless and split methods. Spiking the range of 0.2 to 200 ppm with the same concentration of internal standards eliminated the necessity of preparing two different sets of calibration standards. Table 4 contains the calibration levels of both methods.

A volume of 1 µL of the calibration standards was injected for all methods. Figure 2 shows the chromatogram of the 5 ppm calibration standard acquired in splitless mode.

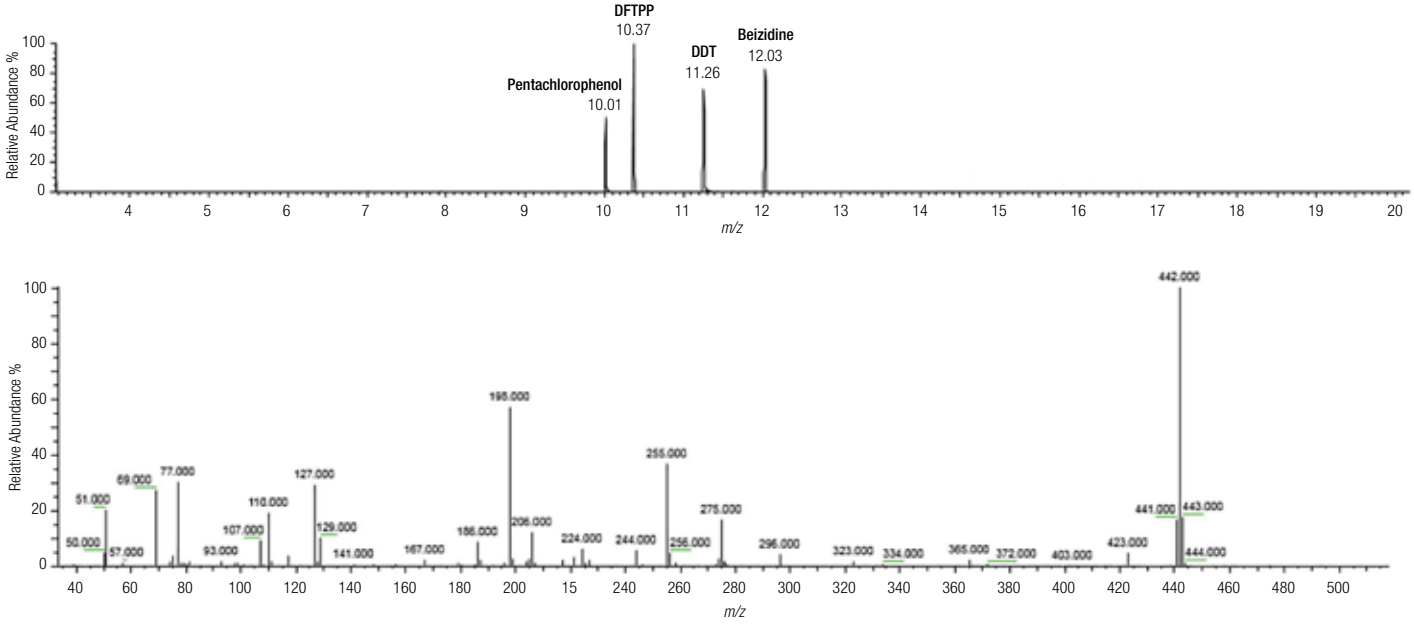

Figure 1. Acquired DFTTP mass spectrum using the ISQ 7000 single quadrupole GC-MS system operated in full-scan at 70 eV ionization energy.

Table 3. DFTPP spectrum check for ion abundance criteria.

Eval Mass (*m/z*)	Ion Abundance Criteria	Measured % Relative Abundance	Criteria Pass/ Fail
51	Greater than or equal to 10% AND less than or equal to 80% of Base Peak	20.7	Pass
68	Less than 2% of *m/z* 69	0.7	Pass
70	Less than 2% of *m/z* 69	0.5	Pass
127	Greater than or equal to 10% AND less than or equal to 80% of Base Peak	29.4	Pass
197	Less than 2% of *m/z* 198	0.1	Pass
198	Greater than 50% AND less than or equal to 100% of Base Peak	57.5	Pass
199	Greater than or equal to 5% AND less than or equal to 9% of *m/z* 198	5.9	Pass
275	Greater than or equal to 10% AND less than or equal to 60% of Base Peak	17.2	Pass
365	Greater than 1% of *m/z* 198	4.6	Pass
441	Greater than 0% AND less than 24% of *m/z* 442	17.4	Pass
442	Greater than 50% AND less than or equal to 100% of Base Peak	100.0	Pass
443	Greater than or equal to 15% AND less than or equal to 24% of *m/z* 442	18.1	Pass

Table 4. Calibration standards used for testing the splitless and split methods.

Calibration Standard	Splitless Conc. (ppm)	Split Conc. (ppm)
Cal 1	0.2	2.0
Cal 2	0.5	5.0
Cal 3	1.0	10.0
Cal 4	2.0	20.0
Cal 5	5.0	35.0
Cal 6	10.0	50.0
Cal 7	20.0	100.0
Cal 8	35.0	200.0
Cal 9	50.0	–

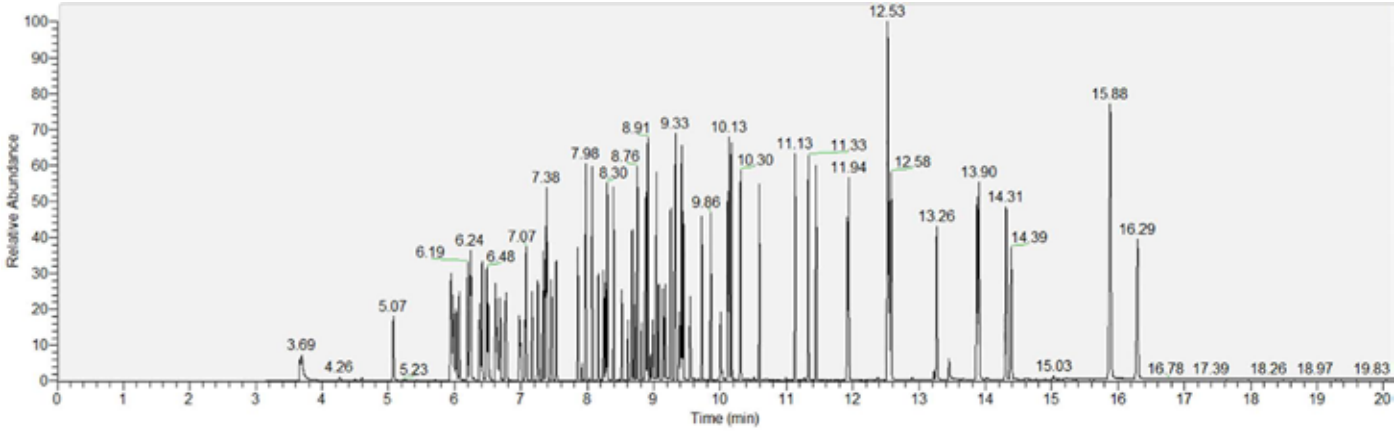

Figure 2. Total ion current (TIC) chromatogram of the 5 ppm EPA 8270 semivolatile calibration standard injected in splitless mode.

Results and discussion

Splitless method 0.2–50 ppm calibration

The average relative response factors of the 76 targeted compounds and six surrogates were calculated by analyzing the nine calibration standards from 0.2 ppm to 50 ppm in methylene chloride. Six compounds had Response Factors %RSD >20% and required an alternative curve fit. The %RSDs of those compounds calibrated using average response factors and r^2 values for the six alternative fit compounds are shown in Table 5.

Split method 2–200 ppm calibration

The average response factors of the 76 targeted compounds and six surrogates were calculated by analyzing eight calibration standards with concentrations ranging from 2 ppm to 200 ppm prepared in methylene chloride. Seven compounds had Response Factors %RSD >20% and required an alternate curve fit. The %RSDs of those compounds calibrated using average response factors and r^2 values for the seven alternative fit compounds are shown in Table 6.

Instant Connect Helium Saver module

Method 8270D was also tested with the Instant Connect Helium Saver module (P/N 19070013). Depending on the experimental conditions, the Helium Saver module allows up to 14 years of GC and GC-MS operation from a single helium cylinder. The inlet is supplied with two different gases: nitrogen is used for the septum purge and split flows with only helium supplying the analytical column. Because of this innovative and patented solution, helium consumption is dramatically reduced.

After time for equilibration, the GC-MS tuning mixture was injected and passed the criteria for EPA Method 8270D. Standards for a calibration curve (0.2–50 ppm and 2–200 ppm) were injected, and the data processed. Table 7a shows the results for splitless method and Table 7b reports split method. In both configurations (SSL and Helium Saver) and for both methods (split and splitless), less than 10% of compounds required an alternative curve fit. All the others had RSD% less than 20% with linear fit.

Minimum response factors

EPA Method 8270D requires a minimum relative response factor (RRF) for any point of the calibration curve for several compounds in the targeted list. Table 8 presents those minimum relative response factor requirements and the minimum RRF across all curves performed on the ISQ 7000 single quadrupole GC-MS system.

Retention times

The four methods: splitless, splitless with Helium Saver, split, and split with Helium Saver, were developed over a period of three weeks. Table 9 demonstrates the stability of the retention times over this period of time. During this time, the liner and septa were changed and the analytical column trimmed. Still, the retention times are reproducible using different methods and different inlet modules. Table 9 shows a comparison of the retention times obtained using different methods and inlet modules.

NeverVent technology

Specifically designed to simplify the routine maintenance procedures and to maximize the GC-MS instrument uptime, the proprietary Vacuum Probe Interlock (VPI) and the V-lock solution available on the ISQ 7000 single quadrupole GC-MS system allow ion source cleaning or column replacement to be performed quickly without breaking the MS vacuum, saving up to 98% of the time typically required to perform those operations. Thanks to the VPI, the ion source can be fully removed—including all of the lenses and the repeller—through the front vacuum interlock, without venting the system. This allows cleaning the source, swapping it, or changing ionization type, and being ready to run samples within minutes, not hours or days. Additionally, the V-lock technology allows the MS under vacuum to be fully isolated from the GC system, permitting not only a quick replacement of the analytical column when necessary, but also quick and safe performance of regular maintenance at the injector side, like replacing the septum or the liner or trimming the analytical column, without the use of any additional post-column or auxiliary gas flow into the MS.

Table 5. Response factors %RSDs as well as coefficient of determination values (r^2) determined from the calibration curve acquired over a concentration range of 0.2–50 ppm (splitless injections).

Compound	%RSD	r^2	Compound	%RSD	r^2
N-Nitrosodimethylamine	11.53	—	Acenaphthylene	8.24	—
Pyridine	10.23	—	1,2-Dinitrobenzene	14.85	—
2-fluorophenol (surrogate)	5.57	—	3-Nitroaniline	8.09	—
Phenol-d6 (surrogate)	4.99	—	**Acenaphthene-d10**	5.78	—
Aniline	6.39	—	Acenaphthene	7.57	—
Phenol	7.30	—	2,4-dinitrophenol	—	0.9867
Bis (2-chloroethyl) ether	7.95	—	Phenol, 4-nitro-	18.15	—
Phenol, 2-chloro-	6.19	—	Dibenzofuran	6.78	—
Benzene, 1,3-dichloro-	6.29	—	2,4-dinitrotoluene	12.32	—
1,4-Dichlorobenzene-d4	4.90	—	Phenol, 2,3,5,6-tetrachloro-	—	0.9957
Benzene, 1,4-dichloro-	7.57	—	Phenol, 2,3,4,6-tetrachloro-	—	0.9965
Benzyl alcohol	7.33	—	Diethyl Phthalate	5.60	—
Benzene, 1,2-dichloro-	7.43	—	4-chlorophenylphenylether	6.50	—
Phenol, 2-methyl-	6.27	—	Fluorene	7.31	—
Bis (2-chloroisopropyl) ether	6.31	—	4-nitroaniline	7.88	—
Phenol, 3&4-methyl-	6.52	—	4,6-Dinitro-2-methylphenol	—	0.9945
N-Nitroso-di-n-propylamine	6.63	—	Diphenylamine	9.61	—
Ethane, hexachloro-	5.80	—	Azobenzene	7.06	—
Nitrobenzene-D5 (surrogate)	5.90	—	2,4,6-tribromophenol (surrogate)	—	0.9963
Benzene, nitro-	3.20	—	4-bromophenylphenylether	4.30	—
Isophorone	3.90	—	Hexachlorobenzene	8.18	—
Phenol, 2-nitro-	13.14	—	Phenol, pentachloro-	—	0.9960
Phenol, 2,4-dimethyl-	4.52	—	Phenanthrene	10.88	—
Bis (2-chloroethoxy) methane	5.17	—	**Phenanthrene-d10-**	3.54	—
Phenol, 2,4-dichloro-	4.76	—	Anthracene	11.38	—
Benzene, 1,2,4-trichloro-	6.17	—	Carbazole	9.69	—
Naphthalene	8.26	—	Di-n-butyl phthalate	8.10	—
Naphthalene-d8	5.02	—	Fluoranthene	10.94	—
p-Chloroaniline	4.95	—	Pyrene	10.68	—
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	5.36	—	p-Terphenyl-d14 (surrogate)	6.76	—
Phenol, 4-chloro-3-methyl-	4.14	—	Benzyl butyl phthalate	8.69	—
Naphthalene, 2-methyl	7.54	—	Bis (2-ethylhexyl) adipate	6.08	—
Naphthalene, 1-methyl-	7.00	—	Benz[a]anthracene	9.68	—
Hexachlorocyclopentadiene	9.80	—	Chrysene	9.38	—
Phenol, 2,4,5-trichloro-	8.21	—	**Chrysene-d12**	4.02	—
Phenol, 2,4,6-trichloro-	5.90	—	Bis (2-ethylhexyl) phthalate	7.42	—
2-fluorobiphenyl (surrogate)	4.99	—	Di-n-octylphthalate	6.30	—
Naphthalene, 2-chloro-	7.24	—	Benzo[b]fluoranthene	6.70	—
2-Nitroaniline	10.43	—	Benzo[k]fluoranthene	8.48	—
1,4-Dinitrobenzene	16.05	—	Benzo[a]pyrene	6.11	—
Dimethyl phthalate	5.66	—	**Perylene-d12**	5.73	—
Benzene, 1,3-dinitro-	13.75	—	Indeno[1,2,3-cd]pyrene	6.36	—
2,6-dinitrotoluene	6.11	—	Dibenzo[a,h]anthracene	6.39	—
			Benzo[g,h,i]perylene	7.75	—

Boldface indicates Internal Standards

Table 6. Response factors %RSDs as well as coefficient of determination values (r^2) determined from the calibration curve acquired over a concentration range of 0.2–200 ppm (10:1 split injections).

Compound	%RSD	r^2	Compound	%RSD	r^2
N-Nitrosodimethylamine	6.31	—	Acenaphthylene	6.59	—
Pyridine	10.80	—	1,2-Dinitrobenzene	15.11	—
2-fluorophenol (surrogate)	4.30	—	3-Nitroaniline	14.42	—
Phenol-d6 (surrogate)	4.19	—	**Acenaphthene-d10**	**7.23**	—
Aniline	4.89	—	Acenaphthene	7.98	—
Phenol	5.48	—	2,4-dinitrophenol	—	0.9984
Bis (2-chloroethyl) ether	4.45	—	Phenol, 4-nitro-	—	0.9982
Phenol, 2-chloro-	4.94	—	Dibenzofuran	8.91	—
Benzene, 1,3-dichloro-	5.03	—	2,4-dinitrotoluene	18.65	—
1,4-Dichlorobenzene-d4	**6.01**	—	Phenol, 2,3,5,6-tetrachloro-	17.58	—
Benzene, 1,4-dichloro-	5.09	—	Phenol, 2,3,4,6-tetrachloro-	12.33	—
Benzyl alcohol	9.21	—	Diethyl Phthalate	7.83	—
Benzene, 1,2-dichloro-	4.76	—	4-chlorophenylphenylether	7.93	—
Phenol, 2-methyl-	6.77	—	Fluorene	9.13	—
Bis (2-chloroisopropyl) ether	4.85	—	4-nitroaniline	13.30	—
Phenol, 3&4-methyl-	5.92	—	4,6-Dinitro-2-methylphenol	-	0.9983
N-Nitroso-di-n-propylamine	6.23	—	Diphenylamine	8.13	—
Ethane, hexachloro-	4.85	—	Azobenzene	9.24	—
Nitrobenzene-D5 (surrogate)	10.59	—	2,4,6-tribromophenol (surrogate)	13.23	—
Benzene, nitro-	10.24	—	4-bromophenylphenylether	6.37	—
Isophorone	5.18	—	Hexachlorobenzene	5.72	—
Phenol, 2-nitro-	19.20	—	Phenol, pentachloro-	—	0.9981
Phenol, 2,4-dimethyl-	4.92	—	Phenanthrene	6.32	—
Bis (2-chloroethoxy) methane	8.67	—	**Phenanthrene-d10-**	**6.95**	—
Phenol, 2,4-dichloro-	5.68	—	Anthracene	7.23	—
Benzene, 1,2,4-trichloro-	5.74	—	Carbazole	11.25	—
Naphthalene	5.74	—	Di-n-butyl phthalate	6.69	—
Naphthalene-d8	**6.53**	—	Fluoranthene	7.64	—
p-Chloroaniline	6.02	—	Pyrene	6.93	—
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	5.54	—	p-Terphenyl-d14 (surrogate)	6.38	—
Phenol, 4-chloro-3-methyl-	8.26	—	Benzyl butyl phthalate	6.97	—
Naphthalene, 2-methyl	6.97	—	Bis(2-ethylhexyl)adipate	6.16	—
Naphthalene, 1-methyl-	7.35	—	Benz[a]anthracene	7.43	—
Hexachlorocyclopentadiene	—	0.9991	Chrysene	6.17	—
Phenol, 2,4,5-trichloro-	10.39	—	**Chrysene-d12**	**10.49**	—
Phenol, 2,4,6-trichloro-	7.92	—	Bis (2-ethylhexyl) phthalate	4.95	—
2-fluorobiphenyl (surrogate)	6.45	—	Di-n-octylphthalate	8.70	—
Naphthalene, 2-chloro-	8.16	—	Benzo[b]fluoranthene	7.06	—
2-Nitroaniline	17.03	—	Benzo[k]fluoranthene	6.26	—
1,4-Dinitrobenzene	—	0.9980	benzo[a]pyrene	6.81	—
Dimethyl phthalate	8.30	—	**Perylene-d12**	**14.99**	—
Benzene, 1,3-dinitro-	—	0.9976	Indeno[1,2,3-cd]pyrene	6.15	—
2,6-dinitrotoluene	11.55	—	Dibenzo[a,h]anthracene	6.91	—
			Benzo[g,h,i]perylene	7.06	—

Boldface indicates Internal Standards

Table 7a. Response factors %RSDs for the 76 targeted compounds and internal standards, as well as r^2, for alternative fit calibrations using the Instant Connect Helium Saver module in splitless mode.

Compound	%RSD	r^2	Compound	%RSD	r^2
N-Nitrosodimethylamine	6.62	—	Acenaphthylene	7.34	—
Pyridine	10.56	—	1,2-Dinitrobenzene	16.57	—
2-fluorophenol (surrogate)	6.37	—	3-Nitroaniline	19.06	—
Phenol-d6 (surrogate)	4.82	—	**Acenaphthene-d10**	**3.99**	—
Aniline	13.52	—	Acenaphthene	4.68	—
Phenol	5.41	—	2,4-dinitrophenol	—	0.9938
Bis(2-chloroethyl) ether	17.24	—	Phenol, 4-nitro-	—	0.9950
Phenol, 2-chloro-	6.34	—	Dibenzofuran	6.21	—
Benzene, 1,3-dichloro-	5.80	—	2,4-dinitrotoluene	—	0.9942
1,4-Dichlorobenzene-d4	**2.53**	—	Phenol, 2,3,5,6-tetrachloro-	—	0.9962
Benzene, 1,4-dichloro-	5.17	—	Phenol, 2,3,4,6-tetrachloro-	14.62	—
Benzyl alcohol	18.38	—	Diethyl Phthalate	5.69	—
Benzene, 1,2-dichloro-	5.36	—	4-chlorophenylphenylether	5.32	—
Phenol, 2-methyl-	6.17	—	Fluorene	9.43	—
Bis(2-chloroisopropyl)ether	4.53	—	4-nitroaniline	19.69	—
Phenol, 3&4-methyl-	7.17	—	4,6-Dinitro-2-methylphenol	—	0.9893
N-Nitroso-di-n-propylamine	7.58	—	Diphenylamine	6.12	—
Ethane, hexachloro-	6.39	—	Azobenzene	6.01	—
Nitrobenzene-D5 (surrogate)	8.67	—	2,4,6-tribromophenol (surrogate)	16.16	—
Benzene, nitro-	8.86	—	4-bromophenylphenylether	8.54	—
Isophorone	5.52	—	Hexachlorobenzene	5.49	—
Phenol, 2-nitro-	17.07	—	Phenol, pentachloro-	—	0.9971
Phenol, 2,4-dimethyl-	8.44	—	Phenanthrene	7.12	—
Bis(2-chloroethoxy)methane	8.87	—	**Phenanthrene-d10-**	**2.95**	—
Phenol, 2,4-dichloro-	8.56	—	Anthracene	12.18	—
Benzene, 1,2,4-trichloro-	5.36	—	Carbazole	6.86	—
Naphthalene	5.91	—	Di-n-butyl phthalate	6.59	—
Naphthalene-d8	**2.41**	—	Fluoranthene	8.46	—
p-Chloroaniline	5.82	—	Pyrene	7.82	—
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	4.82	—	p-Terphenyl-d14 (surrogate)	7.49	—
Phenol, 4-chloro-3-methyl-	8.96	—	Benzyl butyl phthalate	5.81	—
Naphthalene, 2-methyl	5.95	—	Bis(2-ethylhexyl)adipate	9.11	—
Naphthalene, 1-methyl-	6.54	—	Benz[a]anthracene	5.79	—
Hexachlorocyclopentadiene	—	0.9959	Chrysene	6.90	—
Phenol, 2,4,5-trichloro-	13.52	—	**Chrysene-d12**	**4.59**	—
Phenol, 2,4,6-trichloro-	9.81	—	Bis(2-ethylhexyl)phthalate	7.06	—
2-fluorobiphenyl,(surrogate)	6.00	—	Di-n-octylphthalate	7.84	—
Naphthalene, 2-chloro-	5.66	—	Benzo[b]fluoranthene	8.98	—
2-Nitroaniline	17.31	—	Benzo[k]fluoranthene	11.28	—
1,4-Dinitrobenzene	—	0.9962	Benzo[a]pyrene	7.47	—
Dimethyl phthalate	5.88	—	**Perylene-d12**	**5.38**	—
Benzene, 1,3-dinitro-	17.90	—	Indeno[1,2,3-cd]pyrene	8.02	—
2,6-dinitrotoluene	11.80	—	Dibenzo[a,h]anthracene	5.99	—
			Benzo[g,h,i]perylene	7.43	—

Boldface indicates Internal Standards

Table 7b. Response factors %RSDs for the 76 targeted compounds and internal standards, as well as r^2, for alternative fit calibrations using the Instant Connect Helium Saver module in split mode.

Compound	%RSD	r^2	Compound	%RSD	r^2
N-Nitrosodimethylamine	6.62	—	Acenaphthylene	7.25	—
Pyridine	13.09	—	1,2-Dinitrobenzene	17.76	—
2-fluorophenol (surrogate)	6.02	—	3-Nitroaniline	18.05	—
Phenol-d6 (surrogate)	5.71	—	**Acenaphthene-d10**	**4.15**	—
Aniline	6.13	—	Acenaphthene	7.36	—
Phenol	6.52	—	2,4-dinitrophenol	—	0.9965
Bis(2-chloroethyl) ether	5.69	—	Phenol, 4-nitro-	—	0.9978
Phenol, 2-chloro-	7.17	—	Dibenzofuran	6.90	—
Benzene, 1,3-dichloro-	7.28	—	2,4-dinitrotoluene	18.32	—
1,4-Dichlorobenzene-d4	**3.26**	—	Phenol, 2,3,5,6-tetrachloro-	—	0.9957
Benzene, 1,4-dichloro-	8.13	—	Phenol, 2,3,4,6-tetrachloro-	17.05	—
Benzyl alcohol	14.15	—	Diethyl Phthalate	6.09	—
Benzene, 1,2-dichloro-	6.95	—	4-chlorophenylphenylether	8.11	—
Phenol, 2-methyl-	6.68	—	Fluorene	8.51	—
Bis(2-chloroisopropyl)ether	6.28	—	4-nitroaniline	19.17	—
Phenol, 3&4-methyl-	6.42	—	4,6-Dinitro-2-methylphenol	—	0.9987
N-Nitroso-di-n-propylamine	7.31	—	Diphenylamine	7.24	—
Ethane, hexachloro-	9.32	—	Azobenzene	7.28	—
Nitrobenzene-D5 (surrogate)	10.02	—	2,4,6-tribromophenol (surrogate)	14.93	—
Benzene, nitro-	11.59	—	4-bromophenylphenylether	7.06	—
Isophorone	6.70	—	Hexachlorobenzene	7.82	—
Phenol, 2-nitro-	14.78	—	Phenol, pentachloro-	—	0.9991
Phenol, 2,4-dimethyl-	5.90	—	Phenanthrene	8.55	—
Bis(2-chloroethoxy)methane	5.64	—	**Phenanthrene-d10-**	**3.85**	—
Phenol, 2,4-dichloro-	5.96	—	Anthracene	6.87	—
Benzene, 1,2,4-trichloro-	6.67	—	Carbazole	8.99	—
Naphthalene	4.81	—	Di-n-butyl phthalate	7.05	—
Naphthalene-d8	**3.84**	—	Fluoranthene	7.25	—
p-Chloroaniline	5.55	—	Pyrene	6.05	—
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	7.15	—	p-Terphenyl-d14 (surrogate)	6.25	—
Phenol, 4-chloro-3-methyl-	7.32	—	Benzyl butyl phthalate	5.92	—
Naphthalene, 2-methyl	5.92	—	Bis(2-ethylhexyl)adipate	6.32	—
Naphthalene, 1-methyl-	6.15	—	Benz[a]anthracene	7.37	—
Hexachlorocyclopentadiene	—	0.9985	Chrysene	6.90	—
Phenol, 2,4,5-trichloro-	12.06	—	**Chrysene-d12**	**4.81**	—
Phenol, 2,4,6-trichloro-	12.35	—	bis(2-ethylhexyl)phthalate	6.27	—
2-fluorobiphenyl (surrogate)	7.30	—	di-n-octylphthalate	6.56	—
Naphthalene, 2-chloro-	7.68	—	Benzo[b]fluoranthene	6.55	—
2-Nitroaniline	17.72	—	Benzo[k]fluoranthene	9.18	—
1,4-Dinitrobenzene	19.53	—	benzo[a]pyrene	7.40	—
Dimethyl phthalate	7.46	—	**Perylene-d12**	**8.17**	—
Benzene, 1,3-dinitro-	18.89	—	Indeno[1,2,3-cd]pyrene	8.23	—
2,6-dinitrotoluene	13.59	—	dibenzo[a,h]anthracene	7.15	—
			Benzo[g,h,i]perylene	6.50	—

Boldface indicates Internal Standards

Table 8 (Part 1). EPA Method 8270D minimum relative response factors and those produced by the ISQ 7000 single quadrupole system.

Compound	EPA 8270D Minimum Response	Thermo Minimum		Thermo Minimum	
		Splitless	Splitless Helium Saver	Split (10:1)	Split Helium Saver
Phenol	0.8	1.990	2.895	2.603	2.767
Bis(2-chloroethyl) ether	0.7	1.499	2.225	1.929	2.134
Phenol, 2-chloro-	0.8	1.516	1.884	1.882	1.869
Phenol, 2-methyl-	0.7	1.412	1.802	1.719	1.771
Phenol, 3&4-methyl-	0.6	1.495	1.933	1.767	1.897
N-Nitroso-di-n-propylamine	0.5	1.110	1.886	1.254	1.579
Ethane, hexachloro-	0.3	0.530	0.439	0.716	0.690
Benzene, nitro-	0.2	0.316	0.469	0.404	0.471
Isophorone	0.4	0.708	0.989	0.869	0.995
Phenol, 2-nitro-	0.1	0.160	0.170	0.152	0.157
Phenol, 2,4-dimethyl-	0.2	0.389	0.453	0.430	0.465
Bis(2-chloroethoxy)methane	0.3	0.432	0.589	0.530	0.586
Phenol, 2,4-dichloro-	0.2	0.282	0.269	0.313	0.288
Naphthalene	0.7	1.085	1.247	1.176	1.260
p-Chloroaniline	0.01	0.464	0.493	0.497	0.546
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	0.01	0.112	0.118	0.175	0.116
Phenol, 4-chloro-3-methyl-	0.2	0.342	0.394	0.382	0.418
Naphthalene, 2-methyl	0.4	0.785	0.730	0.726	0.724
Hexachlorocyclopentadiene	0.05	0.236	0.128	0.213	0.044
Phenol, 2,4,6-trichloro-	0.2	0.345	0.322	0.372	0.298
Phenol, 2,4,5-trichloro-	0.2	0.324	0.286	0.368	0.300
Naphthalene, 2-chloro-	0.8	1.232	1.388	1.314	1.349
2-Nitroaniline	0.01	0.335	0.406	0.339	0.455
Dimethyl phthalate	0.01	1.361	1.511	1.442	1.482
2,6-dinitrotoluene	0.2	0.229	0.259	0.258	0.242
Acenaphthylene	0.9	1.899	2.216	2.063	2.165
3-Nitroaniline	0.01	0.298	0.336	0.428	0.541
2,4-dinitrophenol	0.01	0.055	0.042	0.045	0.025
Acenaphthene	0.9	1.312	1.574	1.383	1.417

Table 8 (Part 2). EPA Method 8270D minimum relative response factors and those produced by the ISQ 7000 single quadrupole system.

Compound	EPA 8270D Minimum Response	Thermo Minimum		Thermo Minimum	
		Splitless	Splitless Helium Saver	Split (10:1)	Split Helium Saver
2,4-dinitrotoluene	0.2	0.304	0.327	0.316	0.330
Dibenzofuran	0.8	1.840	1.907	1.811	1.863
Phenol, 4-nitro-	0.01	0.167	0.042	0.124	0.055
Diethyl Phthalate	0.01	1.335	1.676	1.508	1.518
4-chlorophenylphenylether	0.4	0.740	0.609	0.692	0.621
4-nitroaniline	0.01	0.306	0.360	0.315	0.296
Fluorene	0.9	1.434	1.647	1.471	1.470
4,6-Dinitro-2-methylphenol	0.01	0.079	0.057	0.063	0.047
Diphenylamine	0.01	0.683	0.897	0.750	0.799
4-bromophenylphenylether	0.1	0.477	0.332	0.241	0.206
Hexachlorobenzene	0.1	0.324	0.256	0.283	0.267
Phenol, pentachloro-	0.05	0.131	0.077	0.064	0.049
Phenanthrene	0.7	1.125	1.335	1.289	1.275
Anthracene	0.7	1.270	1.138	1.272	1.347
Carbazole	0.01	1.070	1.407	1.006	1.156
Di-n-butyl phthalate	0.01	1.314	1.856	1.517	1.626
Fluoranthene	0.6	1.263	1.123	1.268	1.234
Pyrene	0.6	1.072	1.326	1.296	1.487
Benzyl butyl phthalate	0.01	0.496	0.906	0.677	0.847
Bis(2-ethylhexyl)phthalate	0.01	0.741	1.225	0.941	1.144
Chrysene	0.7	1.025	1.110	1.164	1.102
Benz[a]anthracene	0.8	1.068	1.228	1.171	1.124
Di-n-octylphthalate	0.01	1.465	2.673	2.084	2.413
Benzo[b]fluoranthene	0.7	1.364	1.417	1.592	1.432
Benzo[k]fluoranthene	0.7	1.292	1.185	1.586	1.396
Benzo[a]pyrene	0.7	1.353	1.420	1.500	1.414
Indeno[1,2,3-cd]pyrene	0.5	1.600	1.794	1.727	1.866
Dibenzo[a,h]anthracene	0.4	1.393	1.645	1.472	1.617
Benzo[g,h,i]perylene	0.5	1.302	1.560	1.406	1.636

Table 9 (Part 1). Retention times (RT) for the four methods.

Compound	Splitless RT (min)	Split (10:1) RT (min)	Split (10:1) Helium Saver RT (min)	Splitless Helium Saver RT (min)
Pyridine	3.66	3.71	3.66	3.29
N-Nitrosodimethylamine	3.71	3.74	3.68	3.33
2-fluorophenol (surrogate)	5.08	5.07	5.04	4.98
Phenol-d6 (surrogate)	5.96	5.93	5.91	5.92
Phenol	5.97	5.94	5.93	5.92
Aniline	5.98	5.95	5.94	5.92
Bis(2-chloroethyl) ether	6.04	6.00	5.98	5.97
Phenol, 2-chloro-	6.08	6.05	6.03	6.02
Benzene, 1,3-dichloro-	6.20	6.17	6.15	6.14
1,4-Dichlorobenzene-d4	6.23	6.20	6.18	6.17
Benzene, 1,4-dichloro-	6.25	6.21	6.20	6.19
Benzyl alcohol	6.39	6.36	6.34	6.34
Benzene, 1,2-dichloro-	6.42	6.38	6.37	6.36
Phenol, 2-methyl-	6.49	6.46	6.45	6.46
Bis(2-chloroisopropyl)ether	6.51	6.48	6.47	6.46
Phenol, 3&4-methyl-	6.63	6.60	6.59	6.59
N-Nitroso-di-n-propylamine	6.67	6.62	6.60	6.61
Ethane, hexachloro-	6.68	6.65	6.64	6.63
Nitrobenzene-D5 (surrogate)	6.77	6.73	6.72	6.72
Benzene, nitro-	6.79	6.75	6.74	6.74
Isophorone	7.00	6.96	6.94	6.95
Phenol, 2-nitro-	7.06	7.03	7.02	7.02
Phenol, 2,4-dimethyl-	7.09	7.06	7.05	7.06
Bis(2-chloroethoxy)methane	7.18	7.14	7.13	7.13
Phenol, 2,4-dichloro-	7.27	7.23	7.22	7.23
Benzene, 1,2,4-trichloro-	7.33	7.30	7.29	7.29
Naphthalene-d8	7.37	7.34	7.33	7.33
Naphthalene	7.39	7.36	7.35	7.35
p-Chloroaniline	7.46	7.43	7.42	7.42
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	7.53	7.50	7.49	7.49
Phenol, 4-chloro-3-methyl-	7.87	7.84	7.83	7.84
Naphthalene, 2-methyl	7.99	7.95	7.94	7.95
Naphthalene, 1-methyl-	8.08	8.04	8.03	8.04
Hexachlorocyclopentadiene	8.17	8.13	8.12	8.13
Phenol, 2,4,6-trichloro-	8.25	8.21	8.21	8.22
Phenol, 2,4,5-trichloro-	8.28	8.25	8.24	8.25
2-fluorobiphenyl (surrogate)	8.31	8.27	8.26	8.27
Naphthalene, 2-chloro-	8.41	8.37	8.36	8.37
2-Nitroaniline	8.53	8.49	8.49	8.50
1,4-Dinitrobenzene	8.63	8.59	8.58	8.60
Dimethyl phthalate	8.70	8.66	8.64	8.66
Benzene, 1,3-dinitro-	8.74	8.69	8.68	8.70
2,6-dinitrotoluene	8.77	8.72	8.71	8.73
Acenaphthylene	8.77	8.73	8.72	8.73

Table 9 (Part 2). Retention times (RT) for the four methods.

Compound	Splitless RT (min)	Split (10:1) RT (min)	Split (10:1) Helium Saver RT (min)	Splitless Helium Saver RT (min)
1,2-Dinitrobenzene	8.84	8.80	8.78	8.80
Acenaphthene-d10	8.89	8.85	8.84	8.85
3-Nitroaniline	8.90	8.85	8.84	8.86
Acenaphthene	8.92	8.88	8.87	8.89
2,4-dinitrophenol	8.98	8.93	8.92	8.94
Phenol, 4-nitro-	9.02	8.98	8.97	8.99
Dibenzofuran	9.05	9.01	9.00	9.02
2,4-dinitrotoluene	9.10	9.06	9.04	9.06
Phenol, 2,3,5,6-tetrachloro-	9.15	9.11	9.10	9.12
Phenol, 2,3,4,6-tetrachloro-	9.19	9.15	9.14	9.15
Diethyl Phthalate	9.28	9.23	9.22	9.23
4-chlorophenylphenylether	9.33	9.28	9.28	9.29
Fluorene	9.34	9.30	9.29	9.31
4-nitroaniline	9.43	9.38	9.36	9.38
Diphenylamine	9.45	9.40	9.38	9.40
4,6-Dinitro-2-methylphenol	9.45	9.40	9.39	9.41
Azobenzene	9.46	9.42	9.41	9.42
2,4,6-tribromophenol (surrogate)	9.57	9.52	9.51	9.53
4-bromophenylphenylether	9.73	9.69	9.68	9.69
Hexachlorobenzene	9.87	9.82	9.82	9.83
Phenol, pentachloro-	10.02	9.97	9.97	9.98
Phenanthrene-D10-	10.12	10.08	10.07	10.08
Phenanthrene	10.15	10.10	10.09	10.10
Anthracene	10.19	10.14	10.13	10.14
Carbazole	10.32	10.27	10.27	10.28
Di-n-butyl phthalate	10.60	10.55	10.55	10.56
Fluoranthene	11.15	11.10	11.09	11.10
Pyrene	11.35	11.29	11.29	11.30
p-Terphenyl-d14 (surrogate)	11.46	11.40	11.40	11.41
Benzyl butyl phthalate	11.93	11.87	11.87	11.88
Bis(2-ethylhexyl)adipate	11.95	11.89	11.89	11.90
Bis(2-ethylhexyl)phthalate	12.54	12.48	12.47	12.49
Benz[a]anthracene	12.55	12.48	12.48	12.50
Chrysene-d12	12.57	12.50	12.49	12.52
Chrysene	12.61	12.54	12.53	12.55
Di-n-octylphthalate	13.28	13.21	13.20	13.22
Benzo[b]fluoranthene	13.91	13.83	13.82	13.85
Benzo[k]fluoranthene	13.91	13.83	13.85	13.88
Benzo[a]pyrene	14.35	14.26	14.25	14.29
Perylene-d12	14.40	14.32	14.31	14.34
Indeno[1,2,3-cd]pyrene	15.96	15.83	15.81	15.88
Dibenzo[a,h]anthracene	15.96	15.84	15.83	15.88
Benzo[g,h,i]perylene	16.36	16.24	16.23	16.29

Conclusion

The Thermo Scientific ISQ 7000 single quadrupole GC-MS system with the ExtractaBrite ion source and the innovative NeverVent technology is the perfect solution to perform the EPA 8270D Method.

Thanks to the extended dynamic range detection system, the ISQ 7000 GC-MS system allows you to cover a 0.2–200 ppm range with the same column and liner. Seventy-six compounds were reported, and each fulfilled the EPA 8270D requirements in terms of minimum response factors and linearity.

Chromeleon CDS software, with the Environmental Reporting package, offers unparallel flexibility, scalability, and compliance. It provides compliance with EPA 8270D Method requirements offering a full complement of standard reports including DFTPP Tune Check report, Breakdown report, Internal Standard Summary report, Tentatively Identified Compounds report, various quality control reports for check standards, laboratory control samples, matrix spikes, surrogate recoveries, and more.

The Thermo Scientific Instant Connect Helium Saver Module is a unique tool that can be used to reduce the cost per analysis, without compromising the analytical results. The Helium Saver Module makes laboratories more efficient and environmentally friendly, saving 90% of helium during each run.

The ExtractaBrite ion source design, as integrated in the ISQ 7000 GC-MS system, keeps your system cleaner, longer. With heat throughout the ion optics and the patented RF lens, the ISQ 7000 GC-MS system has been proven to be capable to analyze more dirty samples per day, with maximum uptime. Even better, when the instrument finally requires cleaning, the column needs to be replaced or trimmed, or maintenance is required at the injector side, the NeverVent technology offers the user the possibility to operate without venting the MS system, in a very fast and simple way. Why break your workflow when you can have unstoppable productivity?

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thermo scientific

APPLICATION NOTE 10706

Completely cryogen-free monitoring of ozone precursors, air toxics, and oxygenated volatile organic compounds in ambient air in a single run

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Keywords

Gas chromatography, GC, single quadrupole mass spectrometer, FID, flame ionization detector, ISQ 7000, cryogen-free monitoring, PAMS, photo assessment monitoring scheme, TO-15, OVOCs, oxygenated volatile organic compounds, ozone precursors, air toxics, CIA *Advantage*-xr canister autosampler, UNITY-xr thermal desorber, Kori-xr water removal device, Deans Switch, heart-cut, 2D-GC, microfluidics

Goal

To demonstrate using an analytical system comprised of Markes™ CIA *Advantage*-xr™ canister autosampler, Kori-xr™ water removal device, and UNITY-xr thermal desorber coupled to a dual-column Thermo Scientific™ ISQ™ 7000 single quadrupole GC-MS, for the analysis of ozone precursors, air toxics, and oxygenated volatile organic compounds in ambient air.

Introduction

In December 2017, the Chinese Ministry of Environmental Protection issued a document relating to the Environmental Air Volatile Organic Compound Monitoring Program (EA-VOC-MP),[1] which requires the monitoring of 117 compounds comprising three main categories of hazardous airborne volatile pollutants, ozone precursors, air toxics, and oxygenated volatiles compounds:

- Ozone precursors are listed under the U.S. EPA Photochemical Assessment Monitoring Stations (PAMS),[2] and are monitored using either online techniques (for continuous monitoring) or remote canister sampling. Both techniques require water removal and preconcentration of the sample before injection into a GC, usually in a dual column configuration with dual flame ionization detection (FID).[3]

- "Air toxics" are routinely monitored and comprise polar and non-polar VOCs, as well as a number of halogenated compounds. Methodology and performance criteria are detailed in U.S. EPA Method TO-15[4] and Chinese EPA Method HJ 759.[5] Typically, samples are collected in canisters, with water removal and sample preconcentration water taking place prior to injection into a single-column GC-MS system.[6]
- Oxygenated volatile organic compounds (OVOCs): These are a more recent addition to target lists for air monitoring and include a range of aldehydes and ketones. They are typically monitored using derivatization and high-performance liquid chromatography, as specified in Chinese EPA Method HJ 683[7] and U.S. EPA Method TO-11A.[8] However, these protocols require manual processing, the use of solvents, and two analytical platforms, which add significant time and cost to the analysis.

Obtaining good peak shape and chromatographic separation for this combined compound list typically requires cryogenic cooling of the GC column, with the associated cost and inconvenience (in addition, many thermal desorption (TD) systems also require cryogen).

In this study, we demonstrate the quantitative analysis of this challenging 117-compound target list without the use of liquid nitrogen or other cryogen, and with cycle times of less than 60 minutes per sample. The analytical system comprises a canister autosampler, water removal device, thermal desorber, and dual-column GC-MS/FID configured for heart-cut 2D-GC separation. Together, these enable the monitoring of samples at 100% relative humidity, offer optimum responses for the three C_2 and two C_3 hydrocarbon isomers using FID, as well as confident compound identification and high sensitivity for the remaining compounds monitored using MS.

Experimental

Standards

Standard gas cylinders containing 56 PAMS (ozone precursor) compounds (Restek™ 34420) and 65 TO-15 (air toxics) compounds (Restek 34436) and canisters containing five OVOCs listed in TO-11A (formaldehyde, acetaldehyde, hexanal, benzaldehyde, m-tolualdehyde) at 1 ppm in nitrogen were used to prepare standards. Unless otherwise stated, a combined standard at 10 ppb and 100% relative humidity (RH) was used. Thirteen compounds are present in both PAMS and TO-15 standards; therefore, where appropriate, testing was replicated with a single standard to generate accurate data for these compounds. The internal standard comprised bromochloromethane, 1,4-difluorobenzene, chlorobenzene-d_5, and 1-bromo-4-fluorobenzene at 1 ppm in nitrogen (Restek 34408). For reasons of safety in our UK laboratory, (2*E*)-but-2-enal (crotonaldehyde), butanal, propanal, 3-methylbutanal (isovaleraldehyde), and hexanal could not be tested.

Instrument and method setup

The experimental parameters are listed below, and the GC setup is shown in Figure 1, with a schematic explanation of the Deans Switch heart-cut approach. The highly efficient water removal of Markes' cryogen-free Dry-Focus3™ approach allows the GC oven to start at the relatively high temperature of 35 °C.

The analytical system configuration (Figure 2), with a schematic explanation of the Deans Switch heart-cut approach. used for this study was a CIA *Advantage*-xr canister autosampler and UNITY-xr thermal desorber with a Kori-xr water removal device (Figure 3), coupled to an ISQ 7000 single quadrupole GC-MS instrument equipped with an AEI source and coupled to a Thermo Scientific™ TRACE™ 1310 gas chromatograph (Figure 4), in a dual column/microfluidic Deans Switch configuration with dual detection FID/MS.

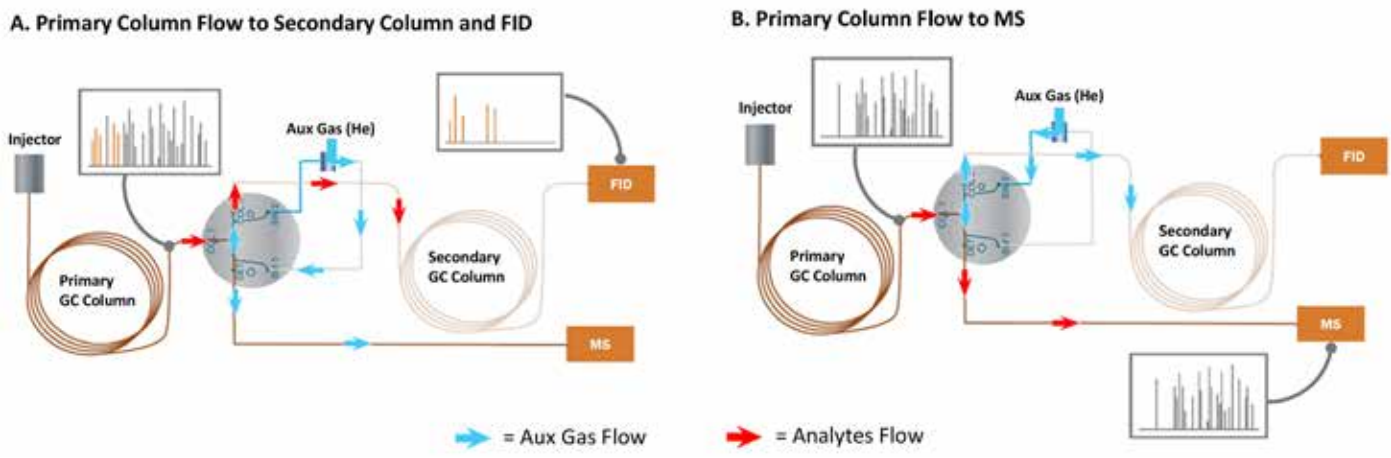

Figure 1. Dual-column GC-MS/FID instrument configuration for Deans Switch 2D-GC operation

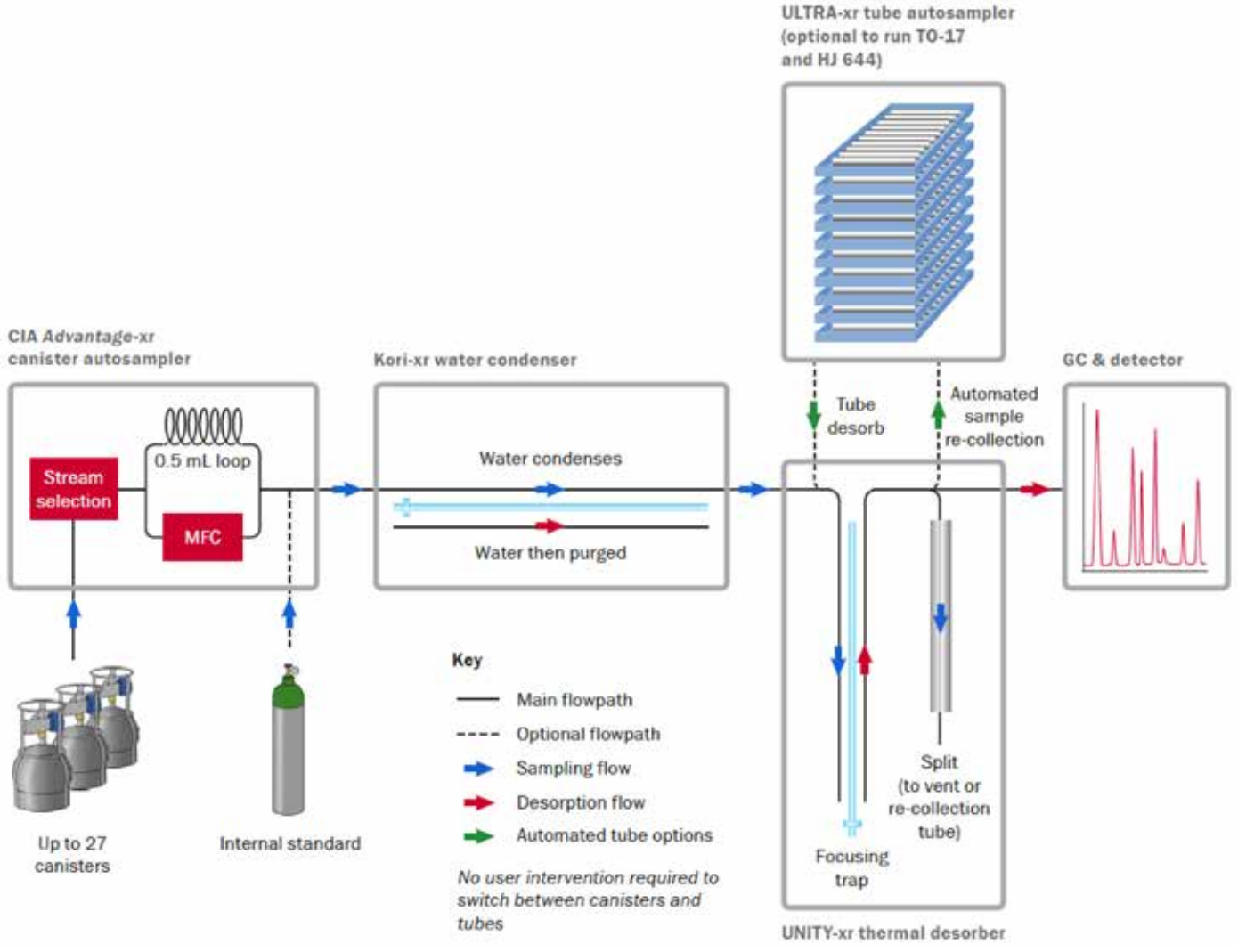

Figure 2. The analytical system configuration used for this study

Figure 3. The CIA *Advantage*–Kori–UNITY-xr system

Figure 4. The Thermo Scientific ISQ 7000 single quadrupole GC-MS instrument equipped with an AEI source and coupled with a Thermo Scientific TRACE 1310 gas chromatograph

The CIA *Advantage*-xr is an autosampler for the analysis of VOCs in canisters or bags, using either a 0.5 mL sample loop or a mass flow controller (MFC). These sampling options allow the automated analysis of both high- and low-concentration samples in a single automated sequence, avoiding the need to resort to dilution of high-concentration samples, and the associated increase in analytical uncertainty and the risk of contaminant introduction. It also overcomes the limitations of traditional cryogen-cooled technology for canister air analysis, such as high costs and flow path blocking caused by ice formation. The CIA *Advantage*-xr also offers internal standard addition via a 1 mL loop, which allows a small volume of a high-concentration internal standard gas to be used, reducing the need for dilution and saving on the consumption of expensive standard gases.

To achieve optimum results for 100% RH ambient air, the amount of residual water reaching the GC-MS system must be very low. For this reason, Markes has developed the Dry-Focus3 approach, as well as a new focusing trap that is optimized for the cryogen-free analysis of VOCs, VVOCs, and oxygenates in humid air.

Ambient air samples first pass through a Kori-xr device that, without use of liquid cryogen, efficiently removes humidity from the air stream while preserving the compounds of interest (Figure 5). With the majority of excess water removed, samples then pass into the trap of the UNITY-xr thermal desorber, held at -30 °C, where the analytes are quantitatively trapped. The trap is then purged with carrier gas in the sampling direction to eliminate oxygen and further reduce water without any loss or breakthrough of the analytes retained. Finally, the flow of gas is reversed, and the trap is heated rapidly (up to 100 °C/s) to inject the analytes onto the GC column.

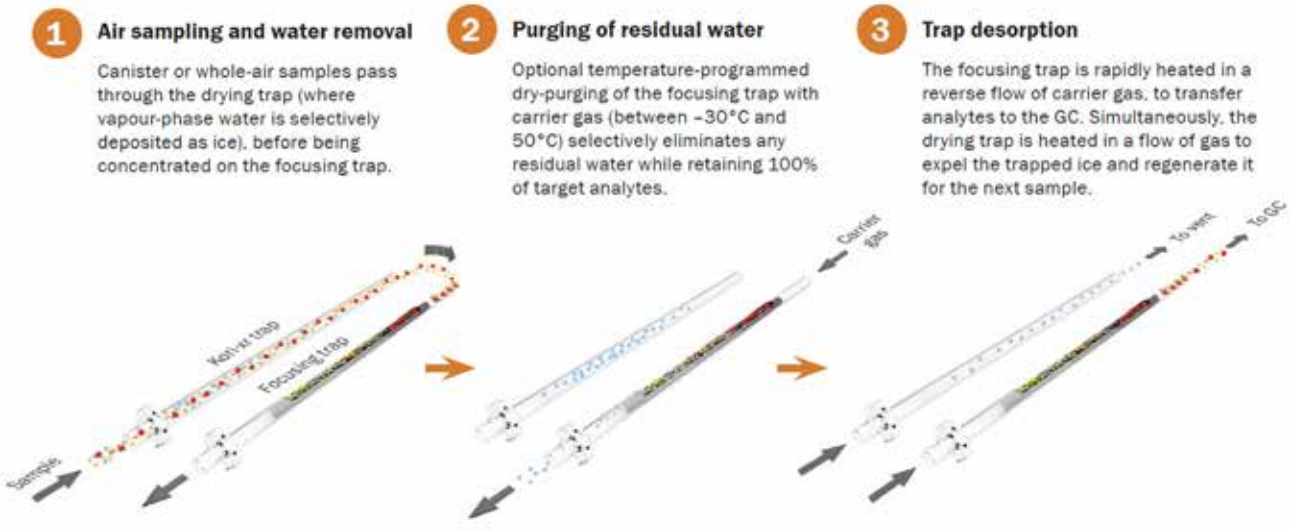

Figure 5. Operation of Dry-Focus3. For an example of the use of trap dry-purging, see Markes Application Note 133.[6]

At this point there is the ability to split the sample, either to vent or onto a clean sorbent tube for storage and re-analysis at a later time (although it should be noted that sorbent tubes are not able to retain very volatile compounds such as acetylene). The above process of sample splitting and re-collection can be fully automated by adding an ULTRA-xr tube autosampler.

The experimental parameters are detailed in Tables 1–4.

Compound separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-VVOC B, 60 m × 0.32 mm I.D. × 5 µm film (P/N 26058-5180) as primary capillary column and a Thermo Scientific™ TracePLOT™ TG-Bond Q+, 30 m × 0.32 mm × 10 µm film (P/N 26005-6030) as secondary capillary column.

Table 1. GC and injector conditions

ISQ 7000 single quadrupole GC-MS instrument parameters					
Inlet temperature (°C):	230				
Carrier gas (mL/min):	He, ramped pressure				
Column flow (mL/min):					
Primary column:	2				
Secondary column:	3				
Inlet module and mode:	SSL, splitless mode				
Purge flow (mL/min):	5				
Primary column:	TraceGOLD TG-VVOC B, 60 m × 0.32 mm I.D. × 5 µm film capillary column (P/N 26058-5180)				
Secondary column:	TracePLOT TG-Bond Q+, 30 m × 0.32 mm x 10 µm film capillary column (P/N 26005-6030)				
Restrictor (to MS):	Fused silica (4.8 m × 0.18 µm)				
Oven temperature program:		*RT (min)*	*Rate (°C/min)*	*Target temperature (°C)*	*Hold time (min)*
	Temperature 1	0	-	35	10.00
	Temperature 2	10	6	240	0.00
	Temperature 3	44	20	270	6
	Run Time	52	-	-	-
Microfluidic Deans Switch		*Time (min)*	*Detector*	*Column*	
device time settings:		0–7.70	FID	Secondary	
		7.70–8.60	MS	Primary	
		8.60–9.44	FID	Secondary	
		9.44–52	MS	Primary	

FID conditions		**MS conditions**	
Temperature (°C):	270	Transfer line (°C):	280
H_2 flow (mL/min):	35	Ionization type:	AEI (EI)
Air flow (mL/min):	350	Ion source (°C):	300
N_2 flow (mL/min):	40	Electron energy (eV):	45
Acquisition rate (Hz):	10 or 25	Acquisition modes:	Full-scan/SIM
Ignition threshold (pA):	1	Mass range (Da):	29–300
Peak width:	Standard	SIM windows:	0–9 min: *m/z* 29; 9–15 min: *m/z* 44

Table 2. Canister sampling conditions

Markes International CIA *Advantage*-xr instrument parameters	
Sample purge (mL/min):	50
Purge time (min):	4
Sample flow:	50 mL/min
Sample volume:	50–600 mL
Post-sample purge:	5 min at 50 mL/min

Table 3. Water removal conditions

Markes International Kori-xr instrument parameter	
Trap temperatures (°C):	–30 °C/300 °C

Table 4. Thermal desorption conditions

Markes International UNITY-xr (Markes International) instrument parameters	
Focusing trap:	Containing a porous polymer, a graphitized carbon black, and a molecular sieve sorbent (Markes P/N U-T22117-2S)
Flow path (°C):	120 °C
Trap purge flow (mL/min):	50
Trap purge time (min):	2
Trap low temperature (°C):	-30 °C
Trap high temperature (°C):	250 °C
Trap high time (min):	2
Outlet split (mL/min):	3

BFB tune

According to the quality requirements of both HJ 759[5] and EA-VOC-MP[1], the GC-MS instrument must be tuned so that 4-bromofluorobenzene (BFB) meets specific criteria for ion abundance (and compliance should be checked before starting a sequence of samples). Table 5 demonstrates that the system used in this study passes the stated criteria for all ions.

U.S. EPA Method TO-15 stipulates that BFB should be injected every 24 hours and the tune criteria assessed. If the system does not pass the acceptance criteria for the BFB tune, corrective action followed by full re-calibration must be performed. Table 5 shows the performance of this system against the BFB tune criteria, demonstrating full compliance of system performance with Method TO-15, with no user intervention.

Data processing

Data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

Results and discussion

Microfluidic Deans Switch device method optimization

Using a dedicated 5-port microfluidic connector for Deans Switch 2D-GC separations (P/N 19005580), optimum sensitivity together with excellent peak shape, retention time stability, and reproducibility were obtained for this complex target list in a single 52-minute chromatographic run. The C_2 hydrocarbons ethene, acetylene, and ethane (which typically require separation on highly retentive columns) respond best to FID detectors, whereas formaldehyde facilitate best to MS detection. It is therefore

Table 5. Results obtained against the BFB tune criteria immediately after tuning

Ion (*m/z*)	Criterion	t = 0 hours (%)	Pass / Fail
50	8–40% of *m/z* 95	16.7	Pass
75	30–60% of *m/z* 95	40.6	Pass
95	Base peak, 100%	100	Pass
96	5–9% of *m/z* 95	8.5	Pass
173	<2% of *m/z* 174	0.7	Pass
174	50–120% of *m/z* 95	86.2	Pass
175	4–9% of *m/z* 174	7.4	Pass
176	93–101% of *m/z* 174	94.1	Pass
177	5–9% of *m/z* 176	6.7	Pass

important to achieve sufficient separation between the C_2 hydrocarbons and formaldehyde to facilitate the first cut to the secondary column. This separation (shown in Figure 6A) was achieved by virtue of a unique combination of optimized TD focusing trap sorbents and a GC oven start temperature of 35 °C. This relatively high initial GC oven temperature is also key to operating this method without the need for liquid cryogen cooling of the GC oven. The C_3 hydrocarbons, like the C_2 hydrocarbons, are also typically detected using FID. This means that after elution of formaldehyde, the primary column flow must be directed back to the FID for propene and propane, with sufficient separation between these and dichlorodifluoromethane to allow the flow to be directed back to the MS again (Figure 6A). Compounds from this point on respond well to the MS detector, enabling them to benefit from the enhanced selectivity. The excellent peak shape and resolution of the C_2 and C_3 hydrocarbons resulting from this double-cut method are shown in Figure 6C, with formaldehyde and dichlorodifluoromethane shown on the MS trace in Figure 6B.

Chromatography and peak shape

Figure 7 shows that good peak shape is obtained across the analyte range, including the least volatile compounds in the list. In addition, the expansion of the 30.5 -31.2 min range demonstrates identification of seven closely-eluting compounds using their extracted ions. It is important to note that the sampling and analysis are achieved within a sample-to-sample cycle time of <60 minutes, maximizing sample throughput without the use of liquid cryogen in the TD or the GC oven. This run time results from a relatively high GC oven starting temperature of 35 °C, available due to the highly efficient water removal of the Markes cyrogen-free Dry-Focus3 and the thermal desorber's overlap mode, in which the next sample is loaded to the focusing trap while the current GC analysis is still running.

Relative response factors and linearities

System linearity was assessed by sampling 50, 100, 200, 300, 400, and 600 mL of the 100% RH, 10 ppb mixed standard. This represents the equivalent mass of each compound that would be sampled from 400 mL of samples with concentrations of 1.25, 2.5, 5, 7.5, 10, and 15 ppb, respectively.

Relative response factors (RRFs) and their relative standard deviations (RSDs) were calculated from the results in accordance with HJ 759 and EA-VOC-MP (Tables A1 and A2, see Appendix). The mean RRF RSD over the six-point calibration was 5% with a maximum of 12%, and therefore well within the 30% limit specified in the methods.

Linearities were also calculated (Tables A1 and A2, see Appendix), and all compounds had R^2 values exceeding the method limit of 0.990, with 93% of the compounds having R^2 values >0.995. Figure 8 shows linearity plots for a selection of compounds covering the volatility and polarity range of the target list.

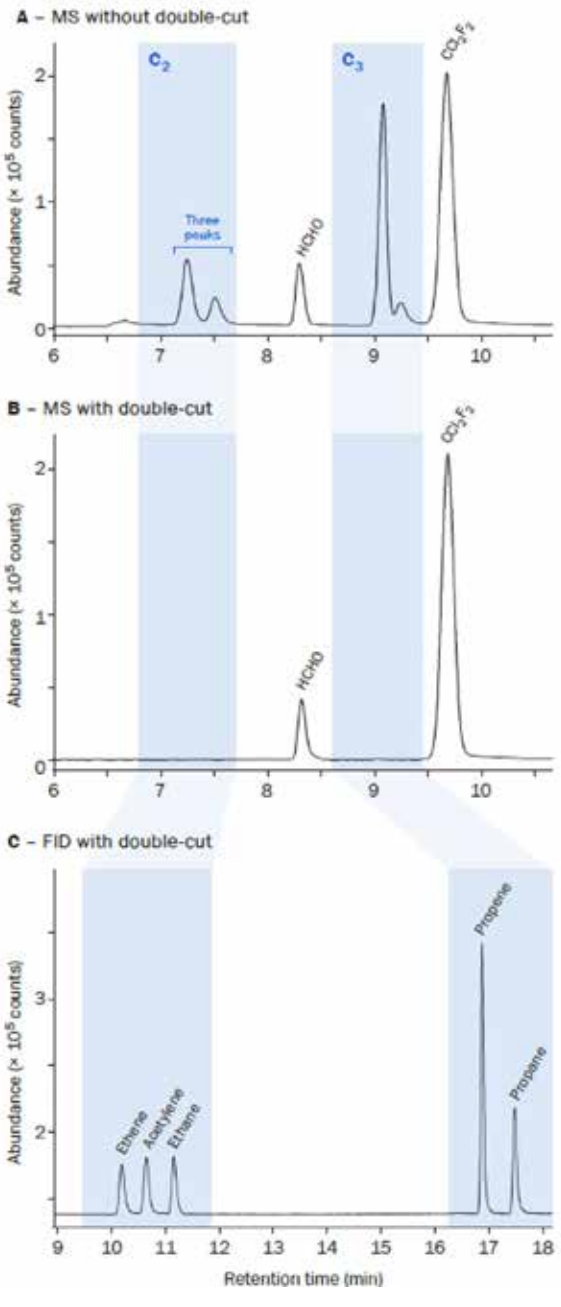

Figure 6. Analysis of 400 mL of the 10 ppb, 100% RH standard, using: (A) Composite MS (primary column) without double-cut, (B) Composite MS (primary column) with double-cut, and (C) FID (secondary column) with double-cut

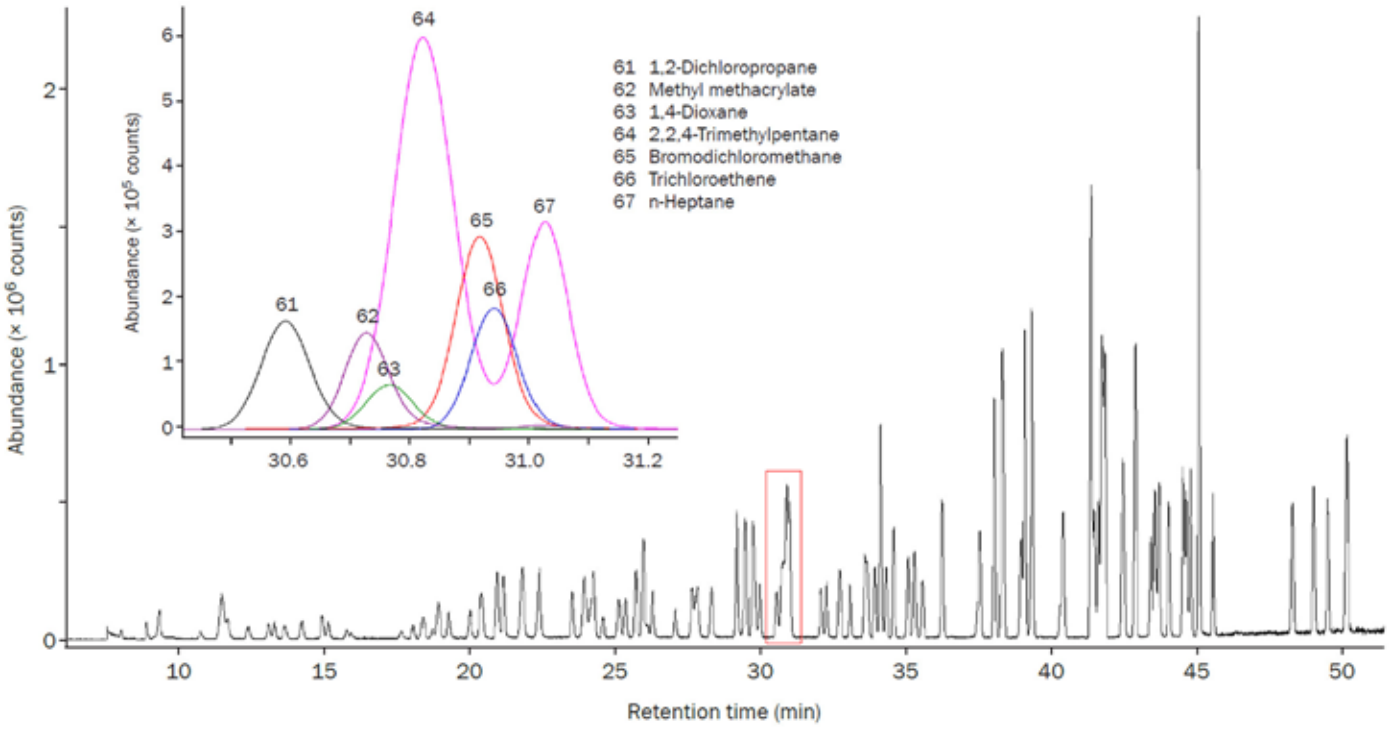

Figure 7. Total ion chromatogram (TIC) of 400 mL of the 10 ppb, 100% RH standard. The inset shows overlaid EIC responses from seven closely eluting analytes in the 30.5–31.2 min region. A full analyte listing is provided in Tables A1 and A2 (see Appendix).

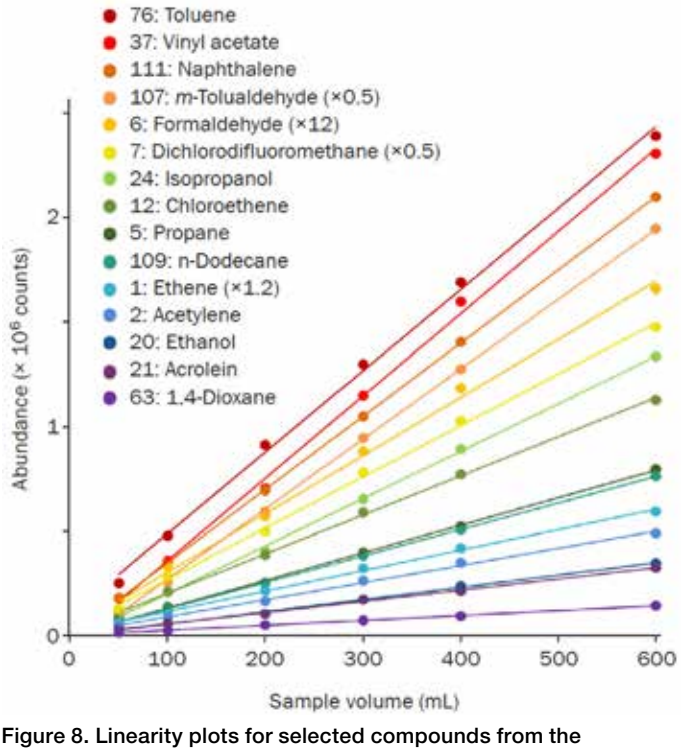

Figure 8. Linearity plots for selected compounds from the 10 ppb, 100% RH standard, over the range 50–600 mL. The scalings indicated have been applied for clarity.

Reproducibility

The nature of the two-column setup means that retention times can be affected by the pressure balance in the system. However, electronic carrier gas control between the GC and the CIA *Advantage*–UNITY-xr, and the efficient removal of water using Dry-Focus3 technology, means that stable retention times are achieved on both columns. Retention-time reproducibility can be expressed as the RSD across a series of analyses, and these values are provided in Tables A1 and A2 (see Appendix).

Excellent retention-time stabilities were achieved over sixteen replicates, with a mean RSD of 0.035% and a maximum of 0.17%—well within the limit of 6% specified in EA-VOC-MP. Such excellent stability of retention times makes it possible to automate the data processing of long sequences of multitarget analyses (for example, like those required by EA-VOCMP), without requiring manual peak integrations or retention time adjustments.

The reproducibility of analyte response was investigated by analyzing ten replicate 400 mL samples at 100% RH. All compounds showed good reproducibility, with <7.5% RSD for all compound areas without the need for internal standard correction. The excellent reproducibility of absolute peak area response and retention time of selected compounds spanning the full range of analytes is shown in Figure 9, and the full list of values can be found in Tables A1 and A2.

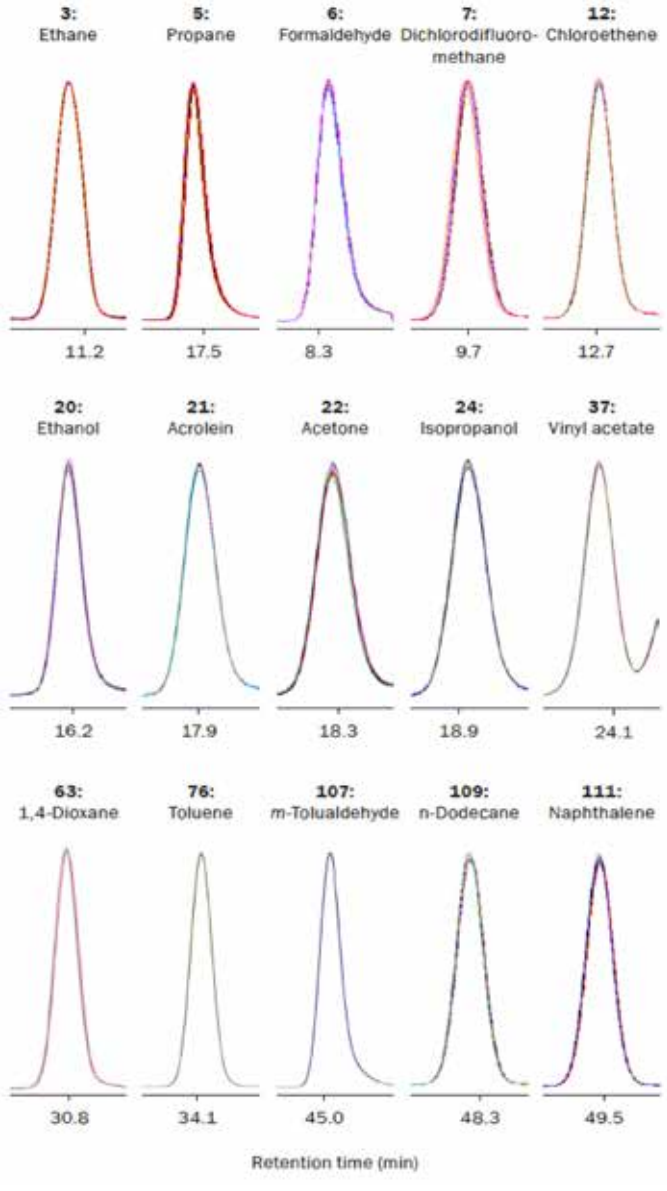

Figure 9. Example overlaid responses (FID for ethane and propane, MS SIM for formaldehyde, MS EIC otherwise) for ten repeat analyses of 400 mL of the 10 ppb, 100% RH standard, demonstrating excellent retention time and response stability

Furthermore, as specified in HJ 759, a gas-phase internal standard (1 mL, 1 ppm) was automatically added to the focusing trap with every sample. Excellent precision was achieved, with all four internal standard compounds yielding absolute response reproducibility <2.1% RSD. This inherent system stability allows confident correction of analyte response across long sequences, which in turn enables the use of the same calibration over an extended period of time, with the obvious benefit of maximizing instrument uptime to run real samples.

In fact, it is important to note that running a complete set of standards in triplicate, at the six concentration levels used in this study, would take approximately 18 hours, so confidence in internal standard response is vital to maintaining high sample throughput.

Confidence in the stability of the internal standard compound responses also allows these compounds to form part of the quality control checks for system performance. As the four-component internal standard is automatically added to every sample, continuous monitoring of the retention time and response of these compounds can provide early warning of changes in the analytical system and reduce the number of external standard quality control samples required throughout the analytical sequence—again increasing the overall laboratory throughput.

Carryover and blank levels

It is important that the instrumentation used for analyzing trace-level samples has minimal memory effects ("carryover"), from previous samples—even if they are at a higher concentration than those typically analyzed. High levels of carryover affect recovery results and require additional blanks to be built into the sequences to prevent any compounds interfering with subsequent samples.

To assess carryover, 400 mL of the 20 ppb, 100% RH standard was analyzed, followed immediately by a 400 mL sample of clean nitrogen. The sample loading in this case represents double the concentration of the highest calibration standard (at the sample volume specified in EA-VOC-MP), and therefore challenges the analytical system with significantly higher concentrations than would be likely in a sampling campaign.

The level of carryover for each compound was quantified both as a percentage of the 20 ppb response (which according to EA-VOC-MP must have a carryover <2.0%), and in terms of the concentration (which must be <0.4 ppb). The majority of compounds were not detected in the carryover test at all, with those that were having a mean value of just 0.028 ppb (0.14%). Figure 10 shows the TIC for the 20 ppb standard, overlaid with the carryover test analysed immediately afterwards. The insets show minimal carryover for both the most and least volatile compounds in the list (formaldehyde and hexachlorobutadiene).

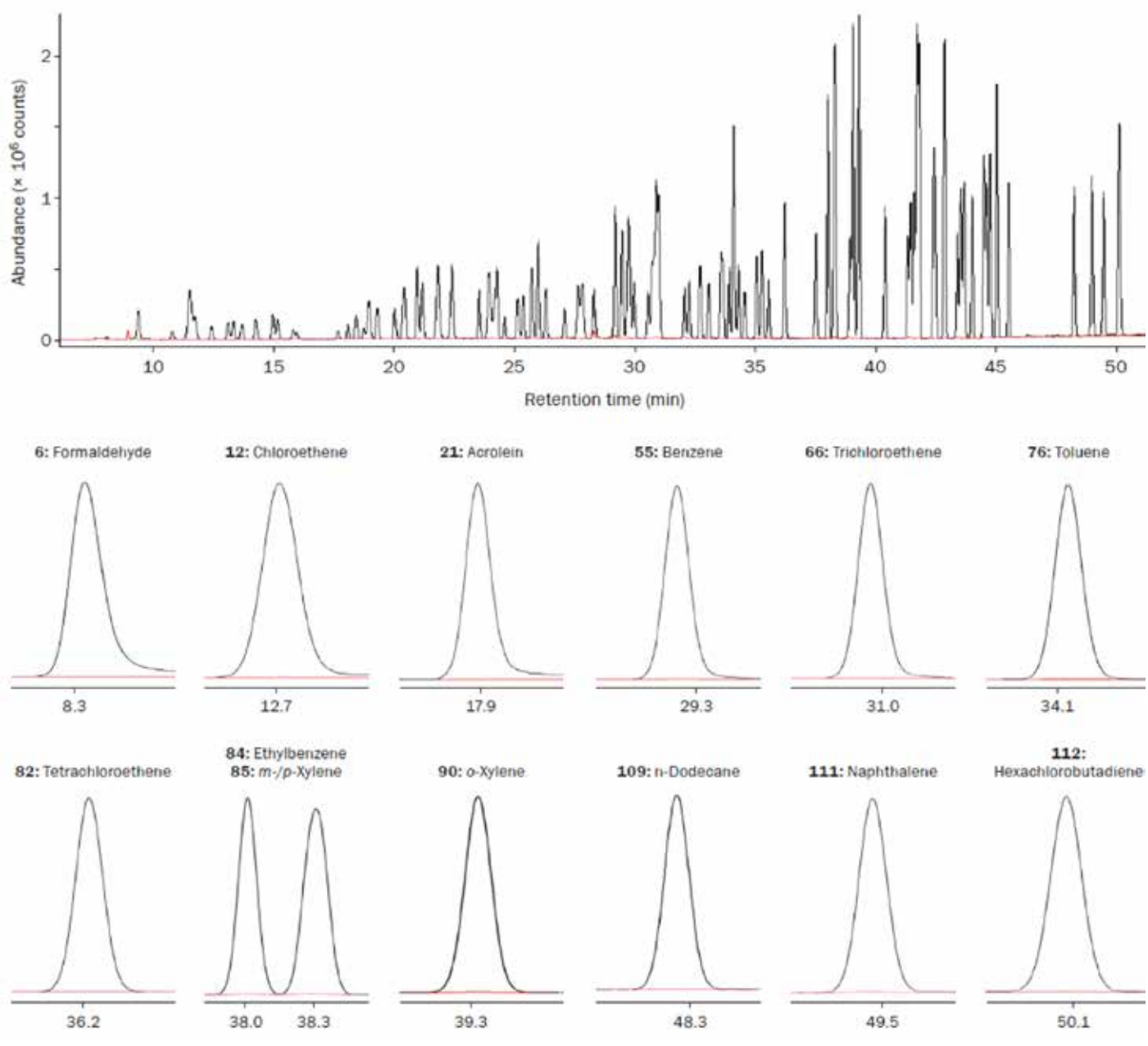

Figure 10. (Top) Analysis of 400 mL of the 20 ppb, 100% RH mixed standard (black) overlaid with a 400 mL nitrogen blank (red), analyzed immediately afterwards to test for carryover. (Bottom) Expansions (MS SIM for formaldehyde, MS EIC otherwise) showing minimal carryover for a range of analytes.

Conclusions

- The CIA *Advantage*–UNITY-xr preconcentration system with Dry-Focus3 technology allows simultaneous, cryogen-free analysis of PAMS ozone precursors, TO-15 air toxics and OVOCs listed in the Chinese Environmental Air Volatile Organic Compound Monitoring Program (EA-VOC-MP).
- The microfluidic Deans Switch two-dimensional GC-MS/FID strategy employed in this work provides confident identification and quantitation, with maximum sensitivity achieved in this challenging application by using the optimum separation and detection system for the various compound types.
- Markes' cryogen-free Dry-Focus3 water management technology has been demonstrated to produce data that satisfies the performance criteria for HJ 759 and EA-VOC-MP for very volatile C_2 hydrocarbons, formaldehyde and acetaldehyde, oxygenated polar VOCs such as acrolein and ethanol, and the less volatile air toxics such as naphthalene, even at 100% relative humidity.
- The analytical system used in the experiments described in this application note provides fully automated analysis for up to 27 sample channels and offers excellent method detection limits, retention time stability, reproducibility and linearity. When combined with the optimised chromatographic method and the overlap mode available (in which the next sample is loaded to the focusing trap while the current GC analysis is still running), sample-to-sample cycle times of less than 60 minutes can be achieved, maximizing laboratory productivity.
- In addition to analyzing the full suite of compounds from canisters, the ability of the CIA *Advantage*-xr to sample from unpressurized sources means that the same instruments can be deployed for remote, unattended, continuous online monitoring of the same compounds with no modifications.
- Additional features of the CIA *Advantage*–UNITY-xr system, are the ability to (a) run sorbent-tube TD analysis in accordance with U.S. EPA Method TO-17 and Chinese EPA Method HJ 644, and (b) re-collect the split portions of samples onto clean sorbent tubes for easier storage and to release the canisters for cleaning and sampling. Moreover, canister and sorbent-tube analyses can be sequenced and run automatically on the same analytical system, without user intervention.

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Appendix

Table A1. Performance data for the compounds from the combined list detected by FID

No.	Compound	t_R (min)	t_R RSD (%) (n=16)	Response RSD (%) (n=10)	R^2	RSD RRF (%)	MDL (ppb)
1	Ethene	10.20	0.120	1.493	0.998	3.6	0.092
2	Acetylene	10.68	0.149	1.860	0.997	3.4	0.099
3	Ethane	11.17	0.101	3.471	0.995	6.6	0.189
4	Propene	16.90	0.095	0.861	1.000	4.4	0.017
5	Propane	17.47	0.092	2.133	0.999	3.3	0.022

Table A2. Performance data for the compounds from the combined list detected by MS

No.	Compound	Mode	t_R (min)	Quant ion (*m/z*)	Qual ion 1 (*m/z*)	Qual ion 2 (*m/z*)	t_R RSD (%) (n = 16)	Response RSD (%) (n = 10)	R^2 (1.25–15 ppb)	RSD RRF (%)	MDL (ppb)
6	Formaldehyde	SIM	8.33	29	—	—	0.073	3.301	0.996	9.8	0.105
7	Dichlorodifluoromethane	EIC	9.70	85	50	—	0.092	6.315	0.998	5.2	0.022
8	Chloromethane	EIC	11.11	50	52	—	0.088	4.577	0.999	6.0	0.095
9	Dichlorotetrafluoroethane	EIC	11.80	85	87	—	0.066	5.489	0.999	5.2	0.034
10	Isobutane	EIC	11.88	43	57	58	0.058	3.427	0.999	4.3	0.022
11	Acetaldehyde	SIM	11.88	44	—	—	0.054	3.171	0.998	10.0	0.019
12	Chloroethene	EIC	12.71	62	35	64	0.054	4.734	0.999	3.8	0.047
13	*trans*-But-2-ene	EIC	13.40	41	39	55	0.040	4.280	0.999	5.0	0.050
14	Butadiene	EIC	13.60	39	53	54	0.045	5.174	0.999	8.6	0.085
15	*n*-Butane	EIC	13.95	43	39	41	0.048	4.342	0.999	4.4	0.060
16	*cis*-But-2-ene	EIC	14.52	41	39	56	0.047	4.256	0.999	6.3	0.059
17	Bromomethane	EIC	15.22	94	96	—	0.035	6.372	0.996	6.1	0.035
18	But-1-ene	EIC	15.41	41	56	39	0.034	4.248	0.999	6.5	0.041
19	Chloroethane	EIC	16.05	64	49	66	0.059	5.041	0.997	6.1	0.050
20	Ethanol	EIC	16.18	31	45	46	0.044	2.154	0.998	9.1	0.043
21	Acrolein	EIC	17.90	56	55	27	0.033	5.495	0.998	4.1	0.032
22	Acetone	EIC	18.28	43	57	42	0.026	5.384	0.999	5.0	0.017
23	2-Methylbutane	EIC	18.62	72	71	—	0.036	4.348	0.999	4.7	0.073
24	Isopropanol	EIC	18.93	45	43	—	0.028	3.131	0.999	8.8	0.114
25	Trichlorofluoromethane	EIC	19.15	101	103	66	0.026	6.046	0.999	4.6	0.037
26	Pent-1-ene	EIC	19.49	42	55	70	0.028	3.954	0.999	8.2	0.083
27	*n*-Pentane	EIC	20.21	43	41	42	0.032	3.949	0.999	6.6	0.062
28	Isoprene	EIC	20.55	67	68	53	0.023	5.141	1.000	3.5	0.057
29	*trans-Pent-2-ene*	EIC	20.61	55	70	42	0.016	4.646	0.999	5.1	0.037
30	1,1-Dichloroethene	EIC	21.12	61	98	96	0.026	4.938	0.999	3.6	0.034
31	*cis*-Pent-2-ene	EIC	21.14	55	42	70	0.023	4.655	0.999	6.1	0.049
32	Dichloromethane	EIC	21.34	49	84	86	0.018	3.032	0.997	3.7	0.099
33	1,1,2-Trichlorotrifluoroethane	EIC	21.97	101	103	151	0.026	6.336	0.998	4.5	0.054
34	2,2-Dimethylbutane	EIC	22.53	43	77	57	0.025	3.838	0.999	4.6	0.066
35	Carbon disulfide	EIC	22.57	76	44	78	0.014	5.653	0.999	4.4	0.045
36	*trans*-1,2-Dichloroethene	EIC	23.67	61	96	98	0.017	4.787	0.999	8.0	0.036
37	Vinyl acetate	EIC	24.04	43	42	86	0.027	5.064	0.999	3.5	0.072
38	*tert*-Butyl methyl ether	EIC	24.06	73	41	57	0.015	5.239	0.998	5.8	0.143
39	1,1-Dichloroethane	EIC	24.15	63	65	83	0.016	4.678	0.997	4.3	0.060
40	2,3-Dimethylbutane	EIC	24.31	43	42	57	0.029	5.237	0.997	10.0	0.080
41	2-Methylpentane	EIC	24.41	42	43	57	0.020	3.407	0.999	3.7	0.062
42	Cyclopentane	EIC	24.40	70	40	55	0.025	5.370	0.999	3.4	0.038
43	Butan-2-one	EIC	24.71	72	57	—	0.021	4.678	1.000	4.2	0.057
44	3-Methylpentane	EIC	25.25	57	41	56	0.022	5.269	0.998	6.7	0.051
45	Hex-1-ene	EIC	25.48	56	41	42	0.024	5.446	0.996	12.1	0.102
46	Ethyl acetate	EIC	25.80	43	45	61	0.022	2.812	0.999	7.1	0.033
47	1,2-Dichloroethene	EIC	25.84	61	96	98	0.021	4.806	0.999	4.2	0.044
48	*n*-Hexane	EIC	26.08	57	41	43	0.013	4.422	0.999	5.8	0.078
IS1	Bromochloromethane	EIC	26.23	130	49	—	0.021	2.904	—	—	—

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Table A2. Performance data for the compounds from the combined list detected by MS (continued from previous page)

No.	Compound	Mode	t_R (min)	Quant ion (*m/z*)	Qual ion 1 (*m/z*)	Qual ion 2 (*m/z*)	t_R RSD (%) (n = 16)	Response RSD (%) (n = 10)	R^2 (1.25–15 ppb)	RSD RRF (%)	MDL (ppb)
49	Chloroform	EIC	26.40	83	47	85	0.021	5.984	0.999	3.1	0.034
50	Tetrahydrofuran	EIC	27.17	42	41	72	0.018	3.469	0.999	5.5	0.084
51	2,4-Dimethylpentane	EIC	27.73	56	41	57	0.025	4.898	0.999	5.8	0.040
52	1,2-Dichloroethane	EIC	27.85	62	49	64	0.020	4.623	0.999	4.7	0.049
53	Methylcyclopentane	EIC	27.93	56	41	69	0.017	4.620	0.995	5.2	0.083
54	1,1,1-Trichloroethane	EIC	28.39	97	61	99	0.021	6.401	0.998	4.9	0.118
55	Benzene	EIC	29.26	78	51	77	0.015	5.579	0.998	3.8	0.014
IS2	1,4-Difluorobenzene	EIC	29.50	114	—	—	0.016	6.012	—	—	—
56	Tetrachloromethane	EIC	29.54	117	119	121	0.015	7.312	0.997	8.5	0.026
57	2-Methylhexane	EIC	29.55	43	42	85	0.013	3.359	0.996	8.4	0.026
58	Cyclohexane	EIC	29.79	84	41	—	0.013	5.609	0.996	7.3	0.008
59	2,3-Dimethylpentane	EIC	29.81	56	43	57	0.018	3.937	0.996	9.3	0.028
60	3-Methylhexane	EIC	30.03	43	57	85	0.016	4.676	0.999	8.2	0.158
61	1,2-Dichloropropane	EIC	30.62	63	41	62	0.012	4.919	0.999	8.3	0.057
62	Methyl methacrylate	EIC	30.75	69	51	89	0.014	4.642	1.000	1.1	0.032
63	1,4-Dioxane	EIC	30.79	88	31	58	0.013	2.563	0.999	5.2	0.120
64	2,2,4-Trimethylpentane	EIC	30.85	57	41	56	0.014	4.102	0.995	6.2	0.033
65	Bromodichloromethane	EIC	30.94	83	47	85	0.017	5.625	0.999	3.2	0.037
66	Trichloroethene	EIC	30.97	130	95	132	0.013	6.668	0.999	6.5	0.029
67	*n*-Heptane	EIC	31.05	57	41	71	0.016	4.722	0.999	8.1	0.068
68	4-Methylpentan-2-one	EIC	32.11	43	41	58	0.012	1.961	1.000	2.4	0.084
69	*cis*-1,3-Dichloropropene	EIC	32.31	75	39	77	0.016	5.182	0.999	3.7	0.018
70	Methylcyclohexane	EIC	32.76	83	41	55	0.015	5.279	0.998	7.8	0.050
71	*trans*-1,3-Dichloropropene	EIC	33.11	75	39	77	0.011	5.001	0.999	2.7	0.046
72	1,1,2-Trichloroethane	EIC	33.62	97	61	83	0.014	5.816	0.998	6.3	0.092
73	2,3,4-Trimethylpentane	EIC	33.70	43	70	71	0.013	3.083	0.997	5.5	0.042
74	2-Methylheptane	EIC	33.96	43	42	—	0.014	3.733	0.997	6.8	0.038
75	Hexan-2-one	EIC	34.12	58	57	—	0.014	1.369	0.998	7.9	0.035
76	Toluene	EIC	34.16	91	65	92	0.012	5.521	0.998	8.0	0.008
77	3-Methylheptane	EIC	34.34	43	41	57	0.010	3.876	0.993	5.7	0.065
78	Hexanal	EIC	34.60	44	56	—	0.012	2.020	0.999	5.9	0.058
79	Chlorodibromomethane	EIC	35.09	129	127	131	0.011	6.661	1.000	1.6	0.051
80	*n*-Octane	EIC	35.30	43	41	57	0.012	3.472	0.997	5.4	0.017
81	1,2-Dibromoethane	EIC	35.59	107	81	109	0.011	5.990	0.999	3.3	0.025
82	Tetrachloroethene	EIC	36.25	166	129	164	0.011	7.337	0.998	7.7	0.032
IS3	Chlorobenzene-d5	EIC	37.45	117	—	—	0.012	5.780	—	—	—
83	Chlorobenzene	EIC	37.54	112	—	—	0.012	6.415	0.998	6.5	0.053
84	Ethylbenzene	EIC	38.03	91	51	106	0.009	5.767	0.998	5.6	0.008
85	*m*-/*p*-Xylene	EIC	38.31	91	105	106	0.011	5.698	0.998	5.4	0.012
86	Bromoform	EIC	38.95	173	171	175	0.010	7.264	1.000	6.3	0.044
87	Styrene	EIC	39.06	104	78	103	0.008	3.198	0.999	4.0	0.041
88	*n*-Nonane	EIC	39.07	43	41	57	0.013	5.835	0.997	4.1	0.005
89	1,1,2,2-Tetrachloroethane	EIC	39.30	83	85	95	0.009	5.037	0.998	4.2	0.071
90	*o*-Xylene	EIC	39.32	91	105	106	0.009	5.753	0.997	6.0	0.007

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Table A2. Performance data for the compounds from the combined list detected by MS (continued from previous page)

No.	Compound	Mode	t_R (min)	Quant ion (*m/z*)	Qual ion 1 (*m/z*)	Qual ion 2 (*m/z*)	t_R RSD (%) (n = 16)	Response RSD (%) (n = 10)	R^2 (1.25–15 ppb)	RSD RRF (%)	MDL (ppb)
IS4	1-Bromo-4-fluorobenzene	EIC	40.28	95	—	—	0.010	5.361	—	—	—
91	Isopropylbenzene	EIC	40.38	105	77	120	0.009	6.163	0.998	4.8	0.025
92	Benzaldehyde	EIC	41.34	106	—	—	0.009	2.928	0.998	11.1	0.078
93	*n*-Propylbenzene	EIC	41.45	91	92	120	0.007	5.424	0.997	3.7	0.037
94	1-Methyl-3-ethylbenzene	EIC	41.72	105	91	120	0.011	4.801	0.997	5.0	0.054
95	1,2,3-Trimethylbenzene	EIC	41.81	119	79	120	0.008	4.285	0.997	3.9	0.037
96	1-Methyl-2-ethylbenzene	EIC	42.40	105	91	120	0.006	6.005	0.997	5.1	0.038
97	*n*-Decane	EIC	42.46	57	41	43	0.007	3.722	0.997	3.2	0.018
98	1,3,5-Trimethylbenzene	EIC	42.85	105	119	—	0.008	6.044	0.998	4.0	0.023
99	1-Methyl-4-ethylbenzene	EIC	42.86	120	77	91	0.034	5.996	0.998	3.5	0.011
100	Benzyl chloride	EIC	43.38	91	126	65	0.009	5.735	0.998	3.3	0.082
101	1,3-Dichlorobenzene	EIC	43.53	146	111	148	0.012	6.636	1.000	2.6	0.078
102	1,4-Dichlorobenzene	EIC	43.66	148	111	75	0.007	6.566	0.999	2.7	0.028
103	1,2,4-Trimethylbenzene	EIC	43.99	105	77	120	0.009	7.136	0.999	2.6	0.031
104	1,3-Diethylbenzene	EIC	44.48	119	134	105	0.010	6.123	0.999	1.8	0.013
105	1,2-Dichlorobenzene	EIC	44.59	146	111	—	0.007	6.760	0.999	2.7	0.025
106	1,4-Diethylbenzene	EIC	44.74	119	105	134	0.009	6.258	0.999	1.5	0.015
107	*m*-Tolualdehyde	EIC	45.03	91	120	—	0.009	1.933	1.000	12.1	0.070
108	*n*-Undecane	EIC	45.50	57	43	71	0.008	2.817	0.999	1.3	0.072
109	*n*-Dodecane	EIC	48.27	57	43	71	0.010	5.050	1.000	1.2	0.073
110	1,2,4-Trichlorobenzene	EIC	48.95	180	145	182	0.012	6.452	1.000	1.6	0.080
111	Naphthalene	EIC	49.43	128	127	129	0.007	5.496	1.000	1.3	0.026
112	Hexachlorobutadiene	EIC	50.08	225	223	227	0.011	7.401	1.000	2.0	0.054
104	1,3-Diethylbenzene	EIC	44.48	119	134	105	0.010	6.123	0.999	1.8	0.013
105	1,2-Dichlorobenzene	EIC	44.59	146	111	—	0.007	6.760	0.999	2.7	0.025
106	1,4-Diethylbenzene	EIC	44.74	119	105	134	0.009	6.258	0.999	1.5	0.015
107	*m*-Tolualdehyde	EIC	45.03	91	120	—	0.009	1.933	1.000	12.1	0.070
108	*n*-Undecane	EIC	45.50	57	43	71	0.008	2.817	0.999	1.3	0.072
109	*n*-Dodecane	EIC	48.27	57	43	71	0.010	5.050	1.000	1.2	0.073
110	1,2,4-Trichlorobenzene	EIC	48.95	180	145	182	0.012	6.452	1.000	1.6	0.080
111	Naphthalene	EIC	49.43	128	127	129	0.007	5.496	1.000	1.3	0.026
112	Hexachlorobutadiene	EIC	50.08	225	223	227	0.011	7.401	1.000	2.0	0.054

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APPLICATION NOTE 667

A comparison between HRAM Orbitrap technology and MS/MS for the analysis of polyfluoroalkyl substances by EPA Method 537

Authors

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Keywords

Contaminants of emerging concern, CEC, perfluorinated compound, perfluoroalkyl acid, PFOA, PFOS, perfluorinated alkyl substances, PFAS, perfluoroalkyl acids, PFAA, persistent organic pollutants, POPs, fire-fighting foam, Orbitrap

Goal

To demonstrate a liquid chromatography – high-resolution, accurate-mass (LC-HRAM) methodology using Orbitrap™ technology as a sensitive, accurate, and reliable quantitative alternative to the use of triple quadrupole mass spectrometers while simultaneously determining unknown perfluorinated compounds in the same drinking water extracts.

Introduction

The unique water-, oil-, grease-, stain- and heat-resistant properties of perfluoroalkyl substances (PFASs) have led to their widespread use in diverse industrial applications and multiple consumer products for over fifty years.

Perfluoroalkyl substances are compounds for which all hydrogens on all carbons (except for carbons associated with functional groups) have been replaced by fluorines, e.g., perfluoroalkyl acids (e.g., PFOA, PFOS). Polyfluoroalkyl substances are compounds for which all hydrogens on at least one (but not all) carbons have been replaced by fluorines, e.g., fluorotelomer-based compounds.[1] The carbon-hydrogen linkages allow for biotic and abiotic degradation in the environment. However, the C–F bond

is considered the strongest single bond in organic chemistry with a bond enthalpy of 481 kJ/mol in CH_3F, which is substantially higher than that of other bonds. This pronounced bond strength is reflected in the notorious environmental and chemical stability of these compounds.[2] (See Figure 1.)

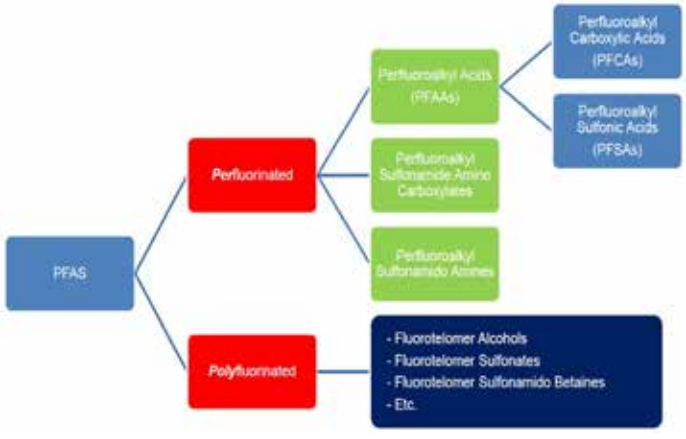


Figure 1. Perfluorinated and polyfluorinated compounds as emerging contaminants in the environment.

The National Institute of Environmental Health Sciences and the National Toxicology Program are supporting research to better understand the potential health effects in humans of exposure to PFASs. In animal studies, some PFASs disrupt normal endocrine activity; reduce immune function; cause adverse effects on multiple organs, including the liver and pancreas; and cause developmental problems in rodent offspring exposed in the womb.[3]

As a result, the United States Environmental Protection Agency (EPA) developed EPA Method 537[4] for the Unregulated Contaminant Monitoring Rule (UCMR 3) program, which collects data for contaminants suspected to be present in drinking water but that do not currently have health-based standards set under the Safe Drinking Water Act (SDWA).[5] In 2012, six PFASs were added to the UCMR 3 list to be monitored, including PFOS and PFOA using EPA Method 537. EPA Method 537 is an offline SPE method using LC-MS/MS detection for the quantitation of linear PFASs in drinking water. In October 2015, occurrence data from the study was released (Figure 2). It is important to note that this is only a small fraction of the hundreds of compounds that can potentially exist in the environment, such as the multiple branched and polyfluorinated PFASs breakdown products that have been known to be in environmental waters. However, standards do not exist for many of these compounds.

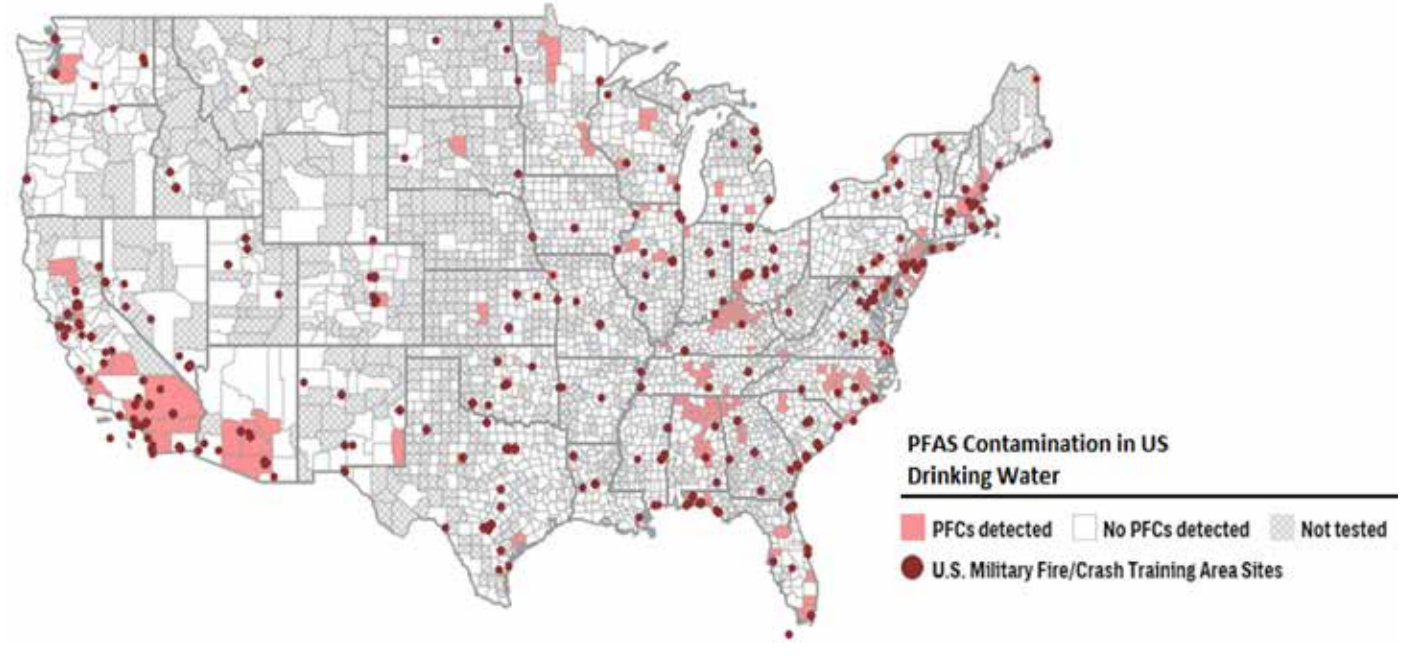


Figure 2. PFAS occurrence data released by EPA for UCMR 3, using EPA Method 537 and monitoring six PFAS compounds.
Data visualization by Moiz Syed. Sources: EPA and Departmennt of Defense. https://theintercept.com/2015/12/16/toxic-firefighting-foam-has-contaminated-u-s-drinking-water-with-pfcs/

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) has been the method of choice for the analysis of PFASs in a variety of matrices. EPA Method 537 is based on this technique, as it allows monitoring of select target analytes in public water supplies. However, other screening strategies taking into account full scan with other advanced MS/MS scan modes can potentially offer a valuable alternative to SRM based methodology due to the development of selective instrumentation for the simultaneous determination of known and unknown contaminants. In addition, high-resolution, accurate-mass (HRAM) capability also provides the ruggedness and sensitivity of MS/MS-based methods without the limitations of unknown identification.

HRAM Orbitrap technology allows for excellent full scan quantitation of target PFASs with MS/MS confirmation. In addition, screening for other contaminants is possible with powerful software tools utilizing comprehensive compound databases and spectral libraries. For this application, we evaluate HRAM Orbitrap quantitation and sensitivity on the Thermo Scientific™ Q Exactive™ mass spectrometer using EPA Method 537 with some minor changes to expand the scope of compounds that can be analyzed using the method. A comparison of HRAM Orbitrap and triple quadrupole mass spectrometry will be described in terms of lowest concentration minimum reporting limit (LCMRL) for the six PFAS compounds in the current EPA Method 537. The results show that HRAM Orbitrap technology provides equal or better quantitation in full scan as compared to traditional triple quadrupole techniques, with the additional capability to screen for unknown PFASs.

Experimental

Sample preparation

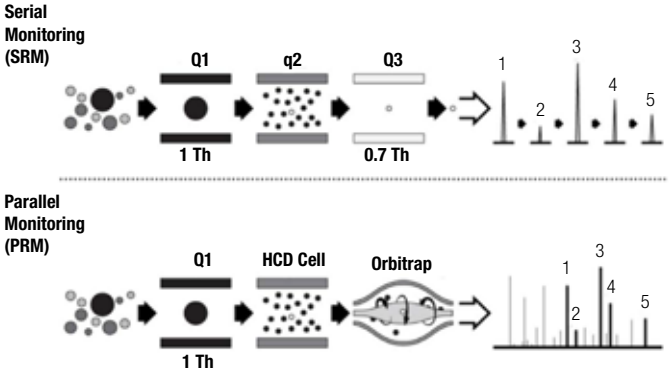
A 250-mL water sample was preserved with Trizma® buffer (MilliporeSigma), fortified with surrogate standards, and passed through a solid phase extraction (SPE) cartridge containing Thermo Scientific™ Dionex™ SolEx™ HRPHS material to extract the method analytes and surrogates. The compounds were eluted from the solid phase with a small amount of methanol. The extract was concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1 mL volume with 96:4% (vol/vol) methanol/water after adding the internal standards. A 5 µL injection was made into an LC equipped with a C18 column that was interfaced to a Q Exactive hybrid mass spectrometer capable of producing full scan and MS/MS data. Note: The use of the modified SPE material mentioned above enabled the capture of smaller PFAS compounds that are beyond the scope of the original EPA Method 537.

Separation

LC:	Thermo Scientific™ UltiMate™ 3000 RS UHPLC system, binary pump, autosampler, and column heater set at 30 °C with 25 µL sample loop
Column:	Thermo Scientific™ Hypersil GOLD™ aQ, 2.1 × 150 mm (3 µm)
Mobile Phase:	A: 20 mM ammonium acetate in water B: Methanol
Gradient:	Start at 30% B, hold for 0.5 minutes and then use a linear gradient to 90% B at 15 minutes, hold for one minute, then drop to original 30% B and equilibrate for additional 3 minutes for a total 19 minutes run time.

Q Exactive MS scan modes and settings

The Q Exactive hybrid mass spectrometer was evaluated using two scan modes: 1) Full scan analysis from *m/z* 100–1000 at mass resolution 70,000 (FWHM) at *m/z* 200, and 2) Parallel reaction monitoring mode (PRM), described in Figure 3, at mass resolution 35,000 (FWHM) at *m/z* 200 and isolation width of 1 Da.



Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics. *Peterson et al., MCP 2012, 0112.020131.*

Figure 3. Parallel reaction monitoring (PRM) in a Q Exactive Orbitrap MS compared to traditional triple quadrupole (serial) MRM analysis.

Full scan acquisition does not require compound optimization for target compounds with the added benefit to perform non-targeted and retrospective data analysis. Accurate quantitation depends upon low ppm mass accuracy and high resolution to discriminate the analyte from matrix components.

PRM is similar to the typical SRM experiment used in a triple quadrupole mass analyzer. The principal difference is that all fragments are collected in a full scan high resolution mass analysis. As a result, multiple MS/MS fragments can be associated with a single precursor. This technique is used for targeted quantitation; thus, retention time, selective compound formula or monoisotopic molecular weight, and collision energy are required to be used in an inclusion list for data acquisition. This experiment empirically has more specificity for the target compound than a full scan experiment since a specific precursor is isolated and fragmented. However, non-targeted analysis is not possible using PRM.

Table 1 describes some key Q Exactive MS settings for each acquisition mode.

Table 1. Q Exactive MS settings.

Full Scan Analysis	
Resolution (FWHM):	70,000
AGC Target:	1.00E+06
Maximum Ion Time:	100 ms
Mass Scan Range:	100–1100 *m/z*
Ion Polarity:	Negative
PRM Analysis	
Resolution (FWHM):	35,000
AGC Target:	2.00E+05
Maximum Ion Time:	100 ms
Isolation Width:	1 Da
Ion Polarity:	Negative

Results and discussion

The liquid chromatography parameters were optimized to ensure good peak symmetry, especially for the early eluting compounds. As the homologous CF_2 backbone increases, the compounds become less polar, exhibiting greater retention on the reversed phase column. The sulfonates are less ionic than those compounds containing carboxylic acids, hence they elute later than PFCA with equal number of carbon atoms in the backbone, e.g., PFOS elutes later than PFOA, although both are C8. Figure 4 displays the observed peak shape and separation obtained with this method.

The sensitivity and linearity for the target compounds on the Q Exactive HRAM Orbitrap mass spectrometer in both the full scan and PRM acquisitions were compared. Example result for PFOA shows comparable sensitivity, specificity, and calibration linearity in both modes (Figure 5). Confirmation of the result in full scan is obtained through isotopic pattern match, retention time confirmation, and mass accuracy (i.e. mass extraction window (MEW)), which is typically 2–3 ppm on the Q Exactive instrument. In PRM mode, a full scan product ion spectrum is obtained and can be used to search against a spectral library. In addition, ion ratio confirmation is possible for further confidence in the identification.

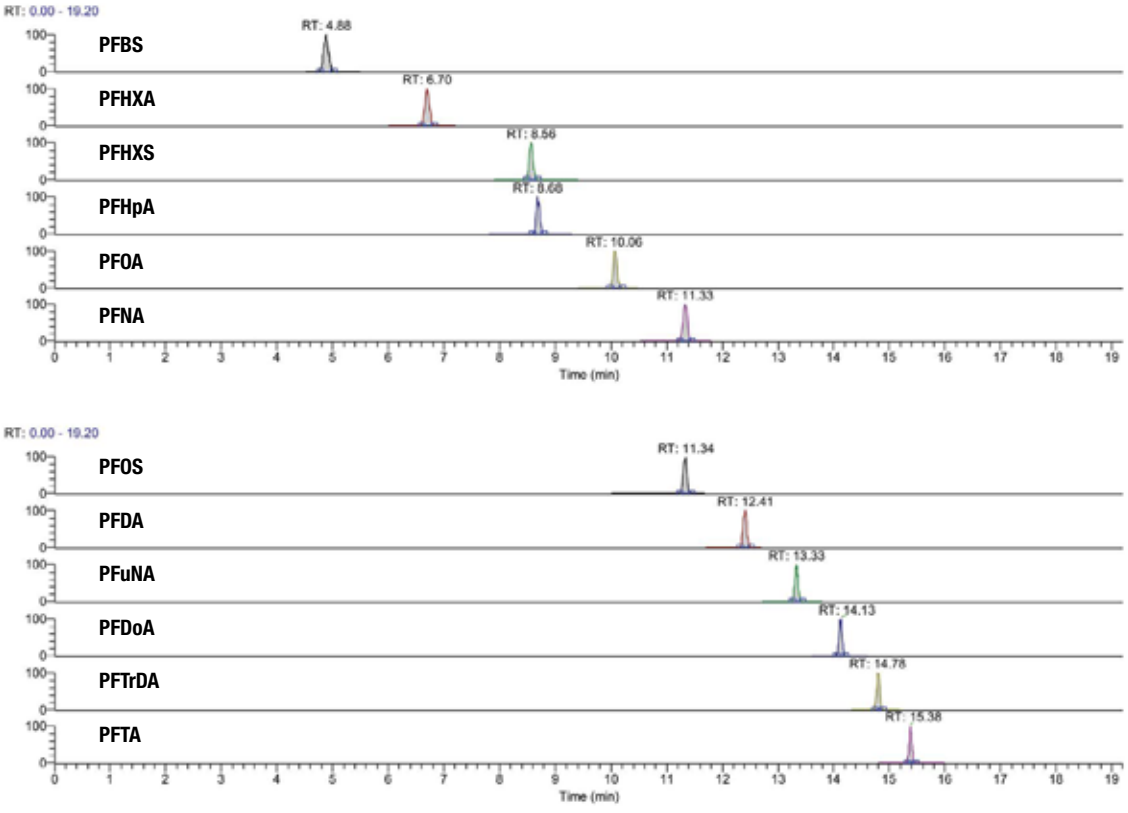


Figure 4. Full scan extracted ion chromatogram of target compounds at 70K resolution, showing good peak shapes and S/N for a 2.5 ppt standard.

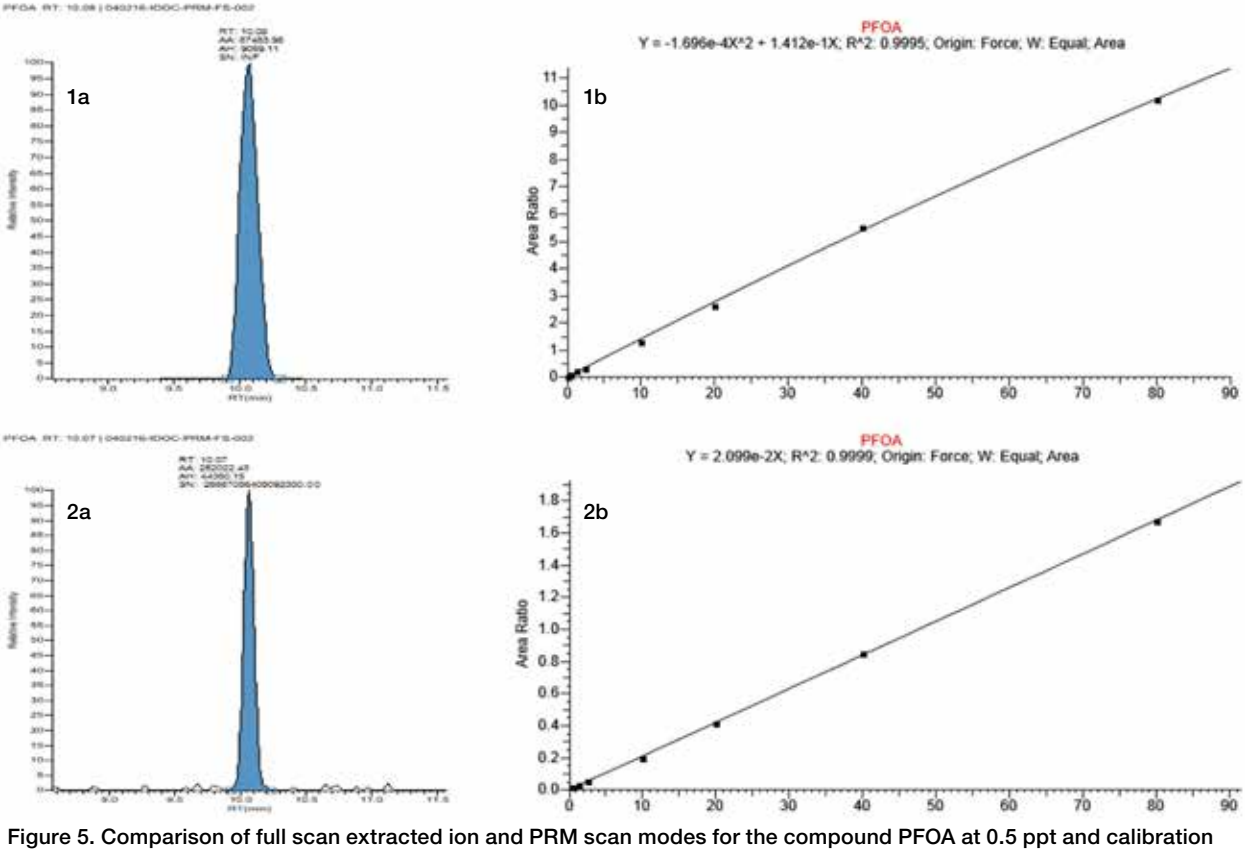


Figure 5. Comparison of full scan extracted ion and PRM scan modes for the compound PFOA at 0.5 ppt and calibration linearity from 0.5 to 80 ppt. (1a, 1b) PRM with primary MS2 transition used for quantitation; (2a, 2b) Full scan extracted ion at 70,000 FWHM used for quantitation.

In Figure 6, a comparison of triple quadrupole SRM, full scan Q Exactive, and PRM Q Exactive analyses is shown for PFOA. Excellent quantitation and sensitivity is obtained with HRAM Orbitrap technology in comparison to QQQ analysis.

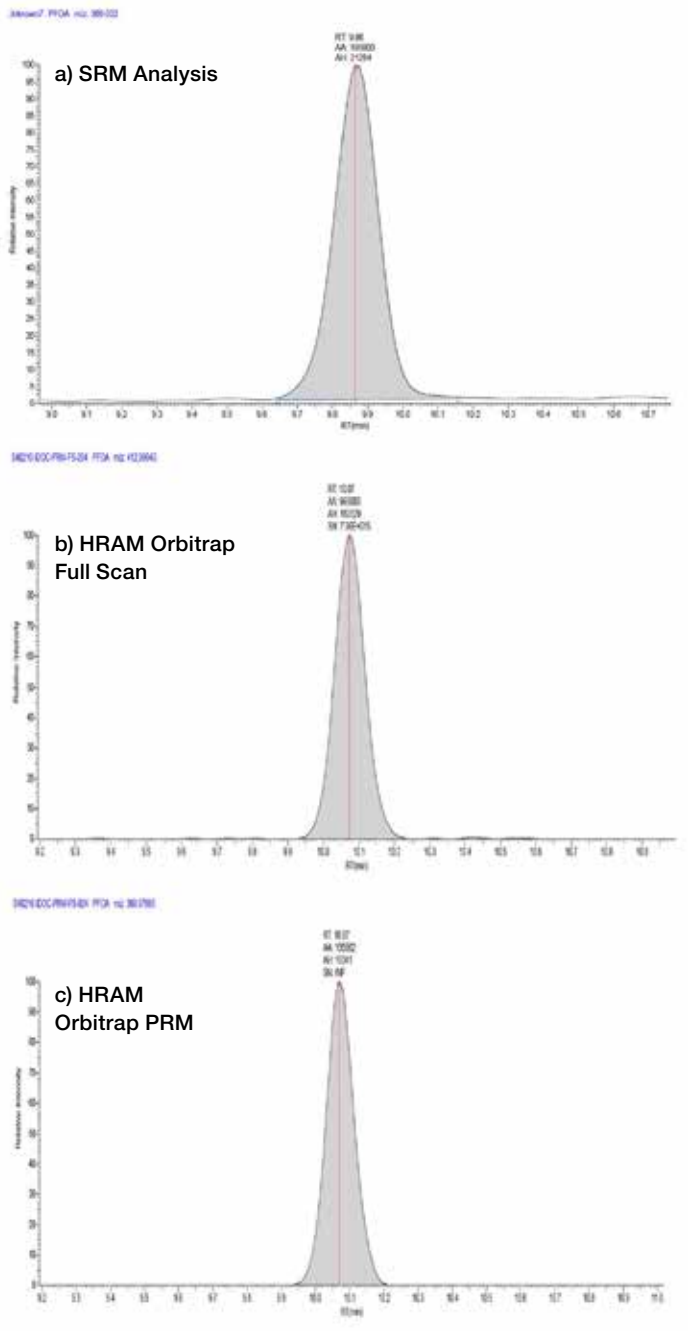


Figure 6. A 2.5 ppt standard of PFOA analyzed by both Q Exactive MS and triple quadrupole mass analyzers under similar conditions (all 5 µL injections). Excellent quantitation and sensitivity is obtained with HRAM Orbitrap in comparison to QQQ analysis.

For EPA Method 537, the lowest concentration minimum reporting limit (LCMRL) is used to evaluate method performance. The LCMRL is defined as the lowest spiking concentration at which recovery of between 50 and 150% is expected 99% of the time by a single analyst. The procedure requires, at a minimum, four replicates at each of seven fortification levels. Four laboratory reagent blanks must also be included. All samples must be processed through the entire method procedure.[6] Test data is entered into a calculator provided by the EPA.

Calculated LCMRLs are shown for both scan modes on the Q Exactive instrument in Figure 7. All results obtained were equal to or better than the published LCMRLs using triple quadrupole SRM analysis for the target analytes in EPA Method 537. Note: the less than values on the LCMRL table means a lower concentration is needed for calculation of LCMRL.

PFOS quantitation

It is important to note that the quantification of environmental samples containing PFOS can be challenging as there is no perfect practical way for accurate quantification of all branched isomers due to different ratios in existing samples and relative response factors. These ratios will differ from calibration standards and between samples from different locations. For PFOS, the 499→99 SRM transition representing a specific branched isomer is generally lower biased relative to the branch representing the SRM transition 499→80 (higher bias). Figure 8 shows a sample containing PFOS compared to a calibration standard. Note that the ratios are not the same, resulting in a biased result if quantitated using EPA Method 537 (the method uses the 499→80 SRM transition). In the Q Exactive instrument, full scan can be used to observe all the branches and appears to be more reliable for quantitation of PFOS. Full scan is closer to the average of the two MRMs and less prone to other factors effecting isomer response factors.

PRM			
EPA Method 537 Target List			
	Critical Level (ng/L)	DL (ng/L)	LCMRL (ng/L)
PFBS	0.077	0.12	<0.5
PFDA	0.18	<0.5	<0.5
PFDoA	0.14	0.29	<0.5
PFHpA		0.35	0.97
PFHxA	0.16	0.27	<0.5
PFHxS		0.52	0.77
PFNA	0.14	0.26	<0.5
PFOA		0.36	0.5
PFOS	0.14	0.21	<0.5
PFTA		0.48	0.71
PFTrDA	0.18	0.32	<0.5
PFuNA		0.31	0.72

Full Scan			
EPA Method 537 Target List			
	Critical Level (ng/L)	DL (ng/L)	LCMRL (ng/L)
PFBS	0.15	0.2	<0.5
PFDA	0.15	0.26	<0.5
PFDoA		0.47	0.73
PFHpA	0.09	0.15	<0.5
PFHxA	0.13	0.19	<0.5
PFHxS		1.7	2.4
PFNA	0.11	0.17	<0.5
PFOA		0.22	0.5
PFOS		0.26	0.5
PFTA	0.15	0.2	<0.5
PFTrDA		0.31	0.55
PFuNA		0.38	1
PFBA		0.19	0.64
PFODA		0.55	1
PFDS	0.13	0.19	<0.5
PFHxDA		0.12	0.5
PFPA	0.18	0.19	<0.5

Figure 7. LCMRL tables for both Q Exactive HRAM Orbitrap scan modes. The compounds highlighted in red are additional analytes that are not part of the original EPA Method 537 list but were found in processed drinking water from the same UCMR3 water extracts.

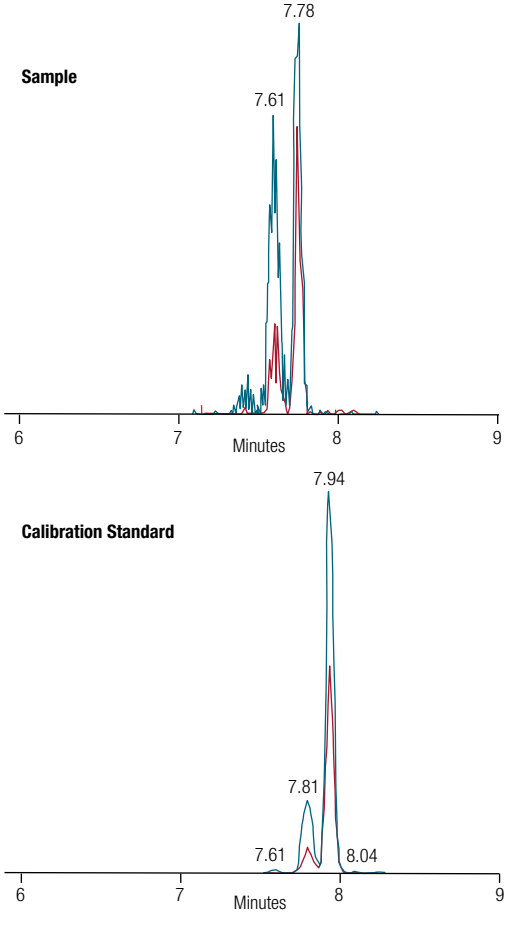


Figure 8. PFOS branch ratio comparison in a sample vs. a calibration standard. These ratios are represented by overlay of the SRM transitions 499→80 (blue trace) and 499→99 (red trace).

Outside of the US, the 499→99 transition is commonly used, whereas EPA Method 537 uses 499→80. The United Nations Environment Program (UNEP) has suggested to take the average of the two using triple quadrupole MS, which makes the results closer to full scan quantitation (Figure 9).

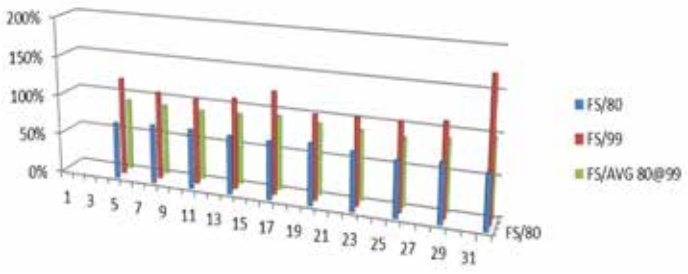


Figure 9. Quantitation comparison of full scan in a Q Exactive MS to SRMs 499→80 and 499→99 (represented as peak area ratios). Results suggest that full scan will have less bias and be close to average of using two SRMs for quantitation.

Screening for other PFASs

As mentioned earlier, an advantage of Q Exactive HRAM Orbitrap instrumentation over targeted analysis using a triple quadrupole MS is the ability to screen for related compounds and other PFASs in samples. For full scan data, retrospective analysis and identification of compounds are possible using spectral libraries, along with retention time and isotope pattern matching for confirmation. Figure 10 shows a sample taken during the UCMR with detection of a non-targeted compound, PFDS using this approach. As predicted, the branched isomer is also detected.

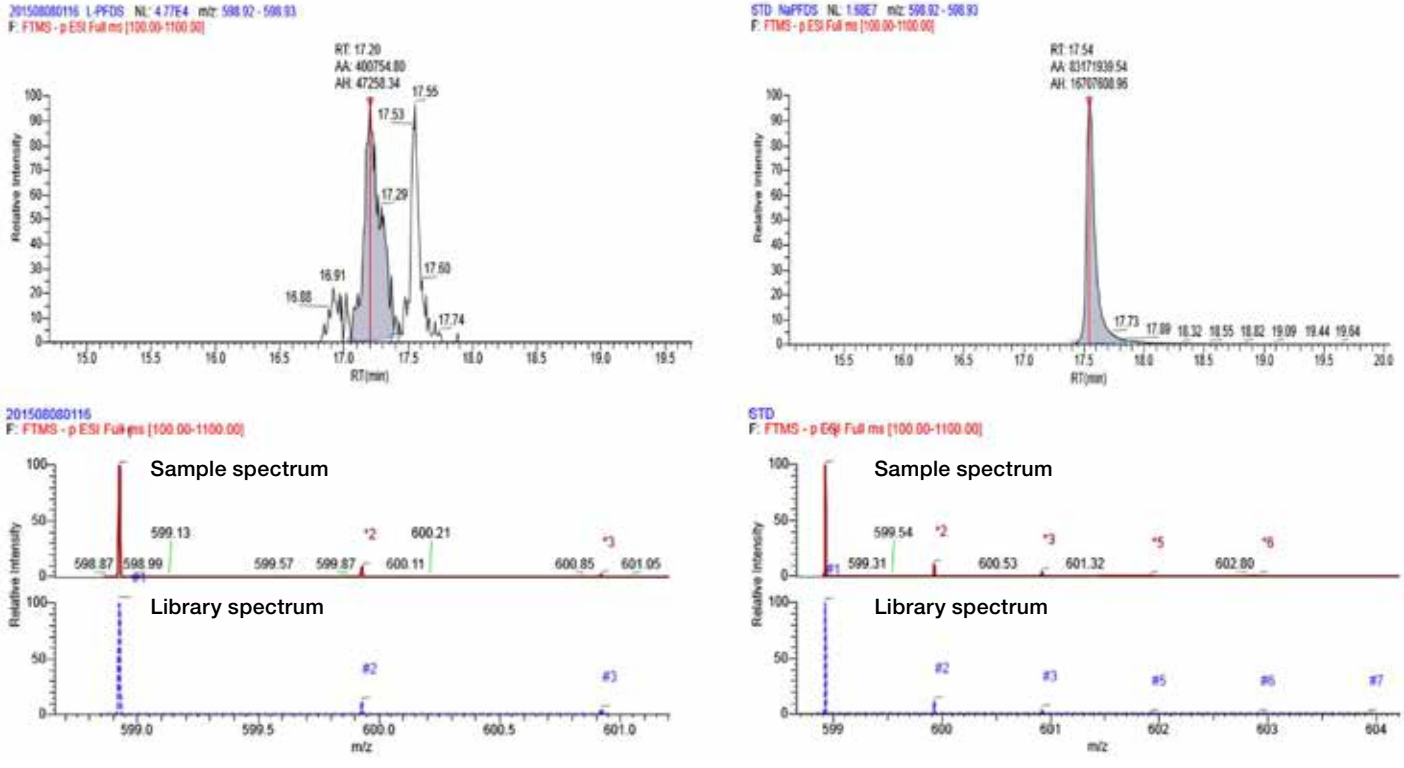


Figure 10. A UCMR3 sample shown having trace hits for a non-targeted compound (PFDS). Post-run identification was performed using an in-house spectral library with isotopic pattern recognition, accurate mass, and retention time for confirmation.

Further interrogation of samples can be performed utilizing a full scan data-dependent acquisition such that both full scan and MS/MS fragments for the top five most intense ions in the mass spectrum are recorded. Powerful data mining tools, such as Thermo Scientific™ Compound Discoverer™ software, allow easy setup of flexible, customized workflows. An example workflow is shown in Figure 11. The software has powerful statistical tools and filters to help narrow down the potential structures of selected compounds, and they can be drawn in a 'custom explanation' using Thermo Scientific™ Mass Frontier™ software to check against accurate mass, isotope pattern, MS, and MS2 data. Known characteristic patterns for suspects can be visualized and used for data filtering. For example, fluorine has a negative mass defect—it has an atomic number of 9 and a relative atomic weight of 18.9984 u. This negative mass defect leads to substantially lower monoisotopic masses of highly fluorinated compounds than the respective nominal mass. Figure 12 is an example of the 'custom explanations' view within the software.

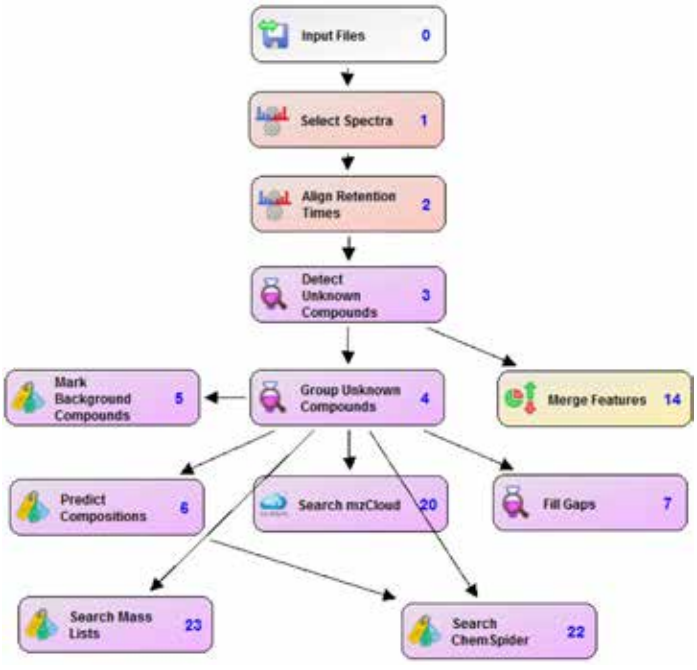


Figure 11. Workflow example in Compound Discoverer software. Flow-chart style elements can be easily dragged and dropped into place for easy customization.

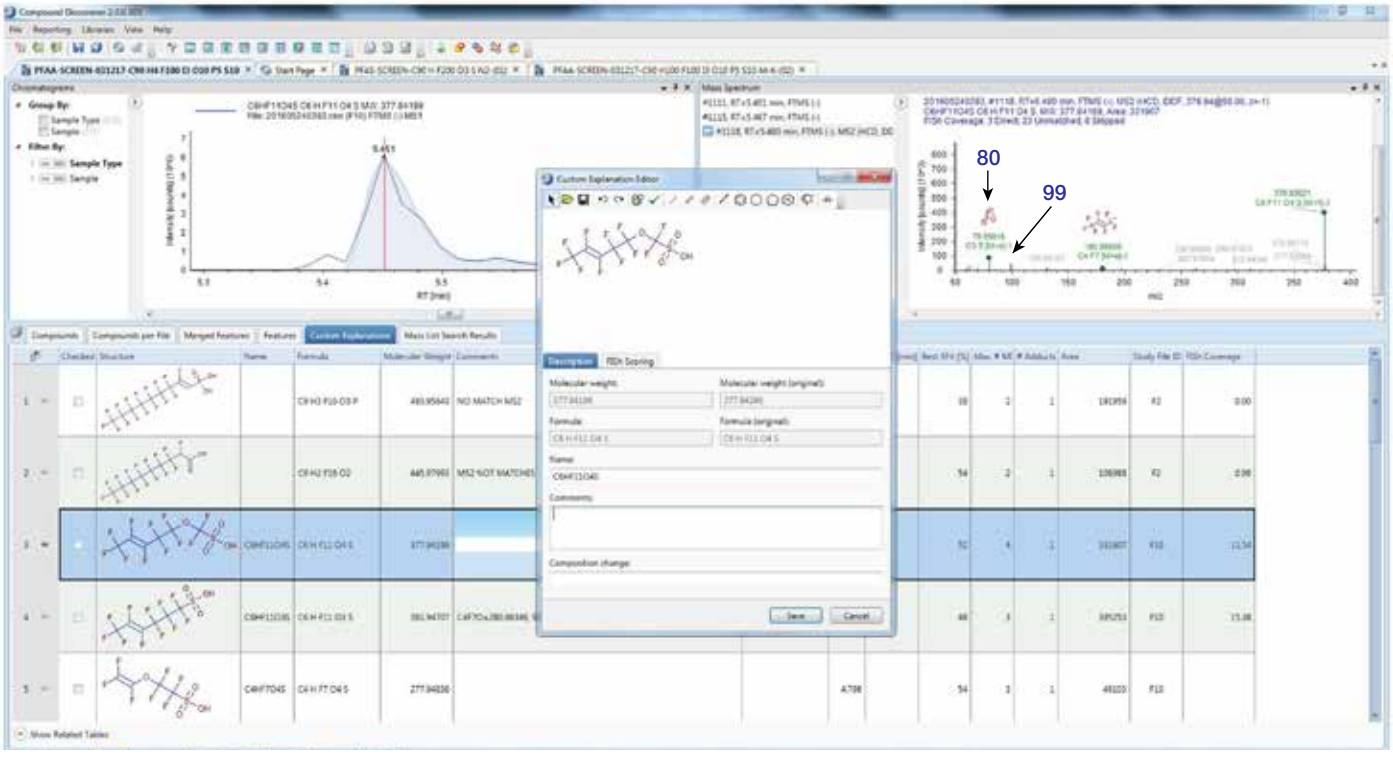


Figure 12. A proposed structure can be drawn in a 'custom explanations' window in Compound Discoverer software using Mass Frontier software for FISH coverage and to check against accurate mass, isotope pattern, with MS2 data displayed in the same workspace.

Conclusion

- Based on the EPA method flexibility rule, QA/QC requirements and guidance within EPA Method 537, HRAM Orbitrap technology should be permissible for potential compliance monitoring if PFASs become regulated compounds in US drinking waters. Q Exactive HRAM Orbitrap instrumentation in the PRM scan mode can be used for quantitation with performance like a triple quadrupole in SRM mode with added specificity, selectivity, and comparable sensitivity.
- Full scan HRAM Orbitrap technology can likely produce more accurate quantitative data for compounds that contain branched isomers such as PFOS.
- Routine quantitative workflows and non-targeted analysis can be performed in a single analysis.
- With complex samples with unknown amounts of other PFASs, utilization of Compound Discoverer software can lower the data processing time and quickly show results.
- Other techniques may be necessary for further confirmation of suspects/unknown structures such as MS^n, ^{13}C, and ^{19}F NMR, when standards are not commercially available.

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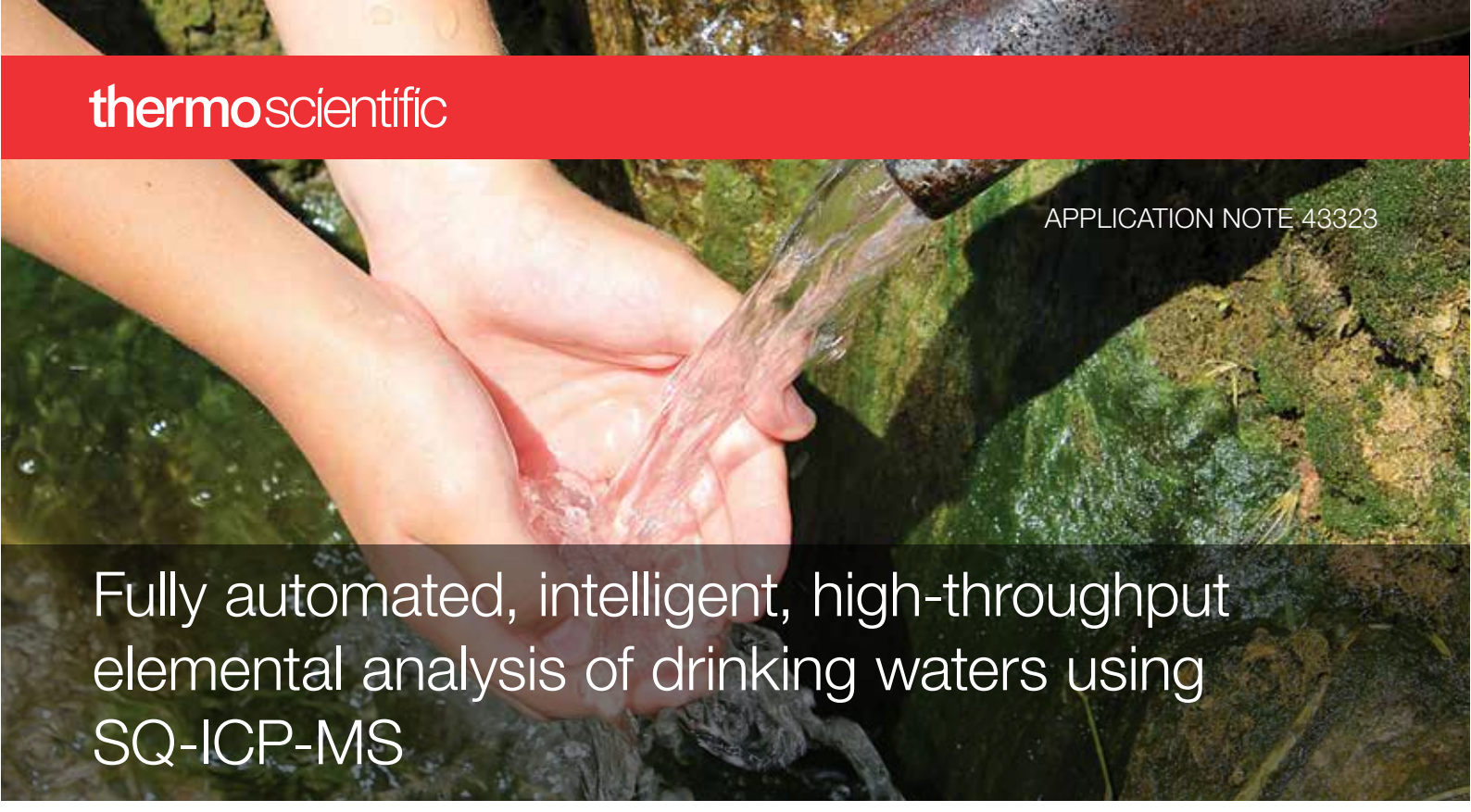

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APPLICATION NOTE 43323

Fully automated, intelligent, high-throughput elemental analysis of drinking waters using SQ-ICP-MS

Authors

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Keywords

Autodilution, Drinking water, EPA method 200.8 revision 5.5, He KED, ICP-MS, Sample preparation, SQ-ICP-MS

Goal

To demonstrate robust high-throughput analysis of environmental samples using SQ-ICP-MS in He-KED mode, in accordance with the requirements of U.S. EPA method 200.8 Revision 5.5 and to demonstrate the performance of the Thermo Scientific™ iCAP™ RQ ICP-MS coupled to the ESI prep*FAST* Autodilution system.

Introduction

EPA Method 200.8 analyses for the quantification of trace metals in drinking and waste waters are performed routinely in many laboratories. Thousands of analyses are performed per week to support the monitoring and control of drinking water contaminants and water quality. Due to the complexity of the standard operating procedure (SOP), skilled technicians are required to setup and prepare the daily analysis, as well as actively monitor the results and perform further sample manipulation as required throughout the analytical run. The need for technical staff is a factor that keeps the overall expense of routinely running the 200.8 method relatively high.

Recent advances in autodilution offer the potential to automate much of the sample preparation and data review with automated re-runs of any samples that do not meet predefined limits. By automatically creating a calibration set of standards from one stock standard and then diluting each sample to a predefined dilution level, an autodilution system can save valuable analysts' time and reduce costs overall through the lowered consumption of utilities and lab supplies.

Fast sample throughput is another driving factor when implementing routine SOPs. Throughput in the method described herein is improved by the discrete sampling of the autodilution system, dramatically reducing uptake and washout time, as well as the use of a single measurement mode for the analysis of all the analytes in the method.

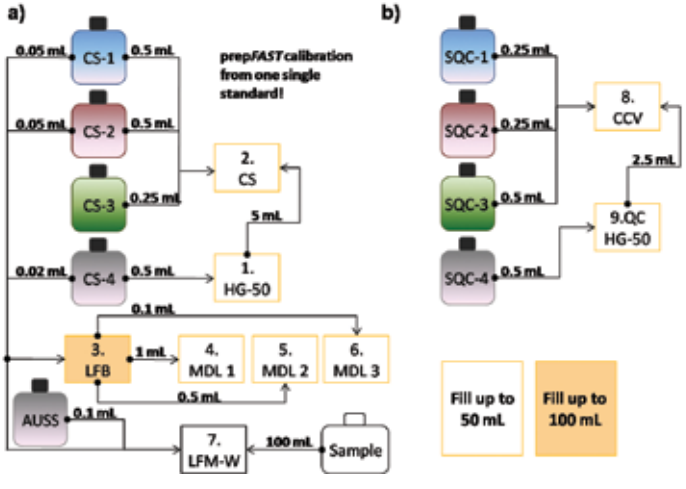
The use of kinetic energy discrimination with helium as a reaction cell gas (He KED) ensures comprehensive interference removal and confidence in the accuracy of the analytical results. Whereas other single quadrupole (SQ) ICP-MS systems require multiple methods for the analysis of drinking water, the iCAP RQ ICP-MS collision/reaction cell (QCell) has a high ion transmission across the mass range so that all of the analytes in the method, including low mass analytes such as Li and Be, can be measured in He KED mode. This eliminates the extra overheads of switching times between different modes and simplifies method development.

This application note describes the fully automated, intelligent, high throughput EPA 200.8 analysis of environmental samples using a prep*FAST* Autodilution system (Elemental Scientific Inc., Omaha, NE, USA) integrated with the iCAP RQ ICP-MS.

Methods

Sample Preparation for U.S. EPA 200.8 Rev 5.5

All samples were prepared according to the EPA 200.8 method. For the determination of dissolved analytes in drinking water, tap water was collected in an HDPE tank and acidified to 1% v/v HNO_3 (Optima™ grade acid, Fisher Chemicals). Aliquots (20 mL) from the tank were filled into 50 mL polypropylene centrifuge tubes for analysis.



AUSS: Gold Standard Solution, CCV: Continuous Calibration Verification, CS-1 to 4: Calibration Standards, HG-50: Mercury Standard (50 ppb), LFB: Laboratory Fortified Blank, LFM-W: Laboratory Fortified Matrix, MDL-1 to 3: Solutions to determine Method Detection Limit, SQC-1 to 4: Standards for Quality control.

Figure 1. Scheme of (a) standard and (b) QC solutions required for EPA 200.8.

The standards and quality control (QC) solutions were prepared according to the protocol outlined in Figure 1.

Mass Spectrometry

The iCAP RQ ICP-MS coupled to the prep*FAST* Autodilution system with an SC-2DX Autosampler (Figure 2) was used for acquisition of all data. The iCAP RQ ICP-MS was operated in He KED mode for all analytes. Instrumental parameters are listed in Table 1.

Table 1. Instrument conditions.

Parameter	Value
iCAP RQ ICP-MS	
Nebulizer	PFA-ST
Nebulizer Gas Flow	1.02 L·min^{-1}
Interface Setup	Ni Cones, High Matrix Skimmer insert
Cell Gas Flow	4.8 mL·min^{-1} He
KED Voltage	3 V
prep*FAST*	
Sample Loop	1.5 mL
Time Per Analysis	66 s

Figure 2. prep*FAST* Autodilution system connected to the iCAP RQ ICP-MS (left). ESI SC-2DX Autosampler (right).

Data Analysis

Thermo Scientific Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software was used for quantitative assessment of the data. Working from a predefined EPA 200.8 template, the only user action needed is to enter the number of samples to be analyzed in the analytical batch. All parameters that must be monitored and achieve certain criteria to comply with EPA 200.8 are automatically checked by the Quality Control feature set included in the default installation of the Qtegra ISDS Software. Samples that do not meet all criteria e.g. Internal Standard (ISTD) recovery rates or over-range analyte concentrations, are automatically diluted to an appropriate level as calculated or defined within the software and the measurement automatically repeated.

Intelligent Autodilution with prep*FAST*

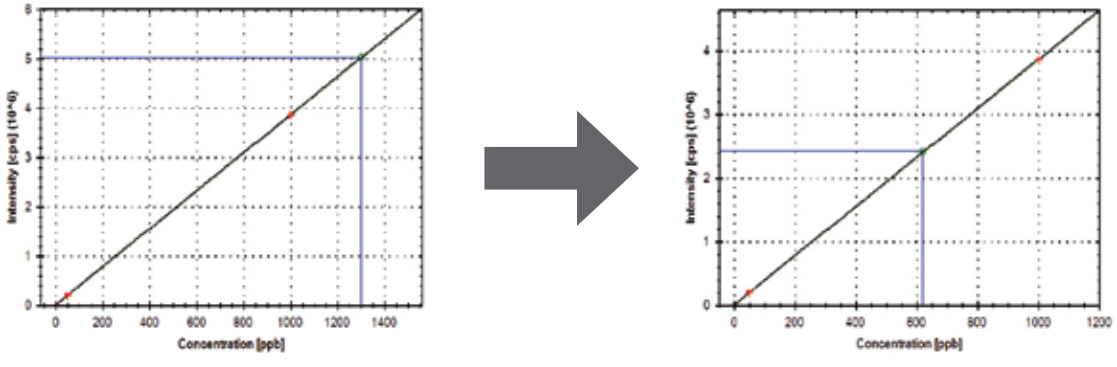
Dilution factors of up to 400-fold are performed reliably and accurately, with all flows controlled by high precision syringe pumps. With the intelligent dilution feature, Qtegra ISDS Software registers every analyte that falls outside of the defined quality control requirements.

If an analyte exceeds the calibration range (Figure 3) the intelligent autodilution dilutes the sample and re-measures only the affected analytes without manual interaction. The applied dilution factor is recorded in the software for full tracability of all dilution steps executed during data acquisition.

Results

Routine Performance of the iCAP RQ ICP-MS

Over 320 tap water samples were analyzed according to method EPA 200.8. The analysis time was, on average, 66 s per sample for the analysis of 21 elements listed in EPA method 200.8 plus 6 different internal standards, leading to a total number of 48 individual isotopes being read out per sample. The concentration of all analytes and their ISTD recovery was monitored throughout the whole analysis time. In total, 508 analyses were run in less than 10 h. Internal standard recovery was well within the EPA 200.8 method requirements of 60 to 125 % (Figure 4).



Analytes exceeding the calibration curve trigger the intelligent auto-dilution!

Measured with corrected dilution factor of 2.165

Figure 3. Analyte concentration re-analyzed by intelligent auto-dilution. Original sample (left), reanalyzed analyte with dillution factor 2.165 (right).

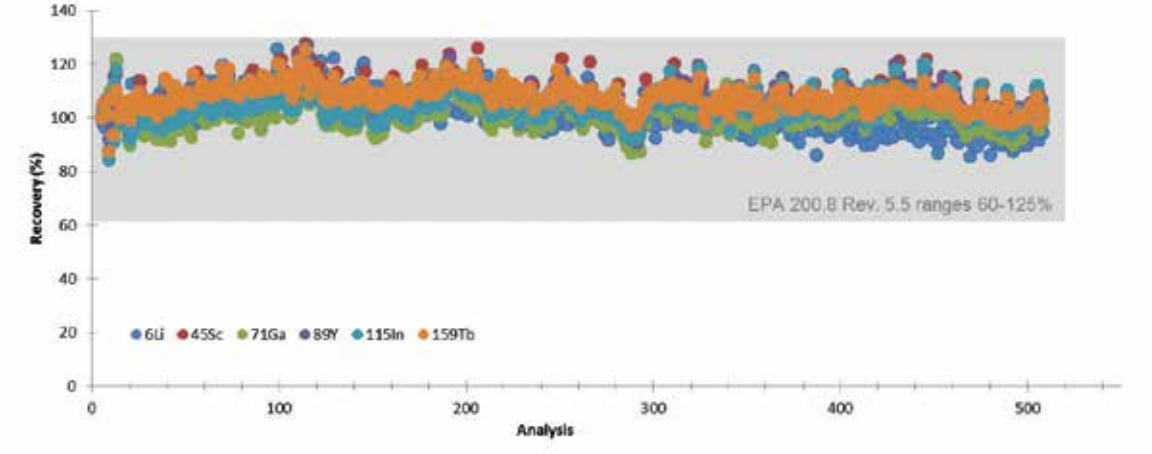


Figure 4. Internal standard response of running tap water samples and QCs showing recoveries well within the 60 – 125% range specified in EPA Method 200.8.

Quality Control (QC) Samples

During the analysis run, a Continuing Calibration Verification (CCV) QC sample was analyzed every 10 samples to assess the accuracy of the calibration throughout the entire batch.

The EPA 200.8 method requires that the recovery of this QC must be within ± 10%, or within the acceptance limits of the method (EPA 200.8, rev 5.5, Table 8). All elements were found to be accurate to within ± 10% of the known concentration, as well as the acceptance criteria, and were stable over all repeated analyses (Figure 5).

Laboratory Fortified Blank and Laboratory Fortified Matrix Recoveries

The recovery of a Laboratory Fortified Blank (LFB) with known added amounts of analytes (Figure 1a, solution 3) must be measured at least once per batch of samples. During this assessment, the LFB was analyzed 32 times and the calculated recovery rates are shown in Figure 6. All analytes show recoveries within the limits (85–115%) of EPA 200.8. Similar to the LFB recovery for every batch, one sample must also be spiked with a known amount of analytes, (Laboratory Fortified Matrix sample; LFM). All 32 LFM (Figure 1a, solution 7) samples were within the EPA 200.8 recovery limits (75-130%).

Driven by Qtegra ISDS Software

Fully Integrated

The Qtegra ISDS Software provides all required features needed for the high throughput analysis of environmental samples. Together with the fully integrated prep*FAST* Autodilution system, Qtegra ISDS Software offers:

- Prescriptive dilution of samples and calibration standards.
- Continuous monitoring of all quality controls (LFB and LFM recoveries or duplicate sample verification)
- LabBook feature that starts an intelligent sequence, with full QA/QC protocols, and subsequently processes and reports results.
- Comprehensive, user definable reports enabling flexible export to external LIMS software packages.

Intelligent autodilution for samples exceeding the calibration range is fully integrated. Samples re-measured by the Qtegra ISDS Software are added automatically to the sample list and clearly identified by a plus sign (Figure 7).

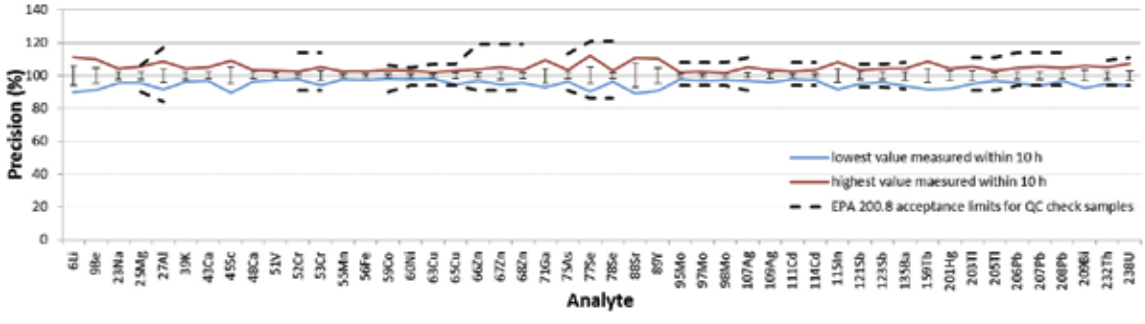


Figure 5. QC recovery and stability of the continuous calibration samples over the entire batch.

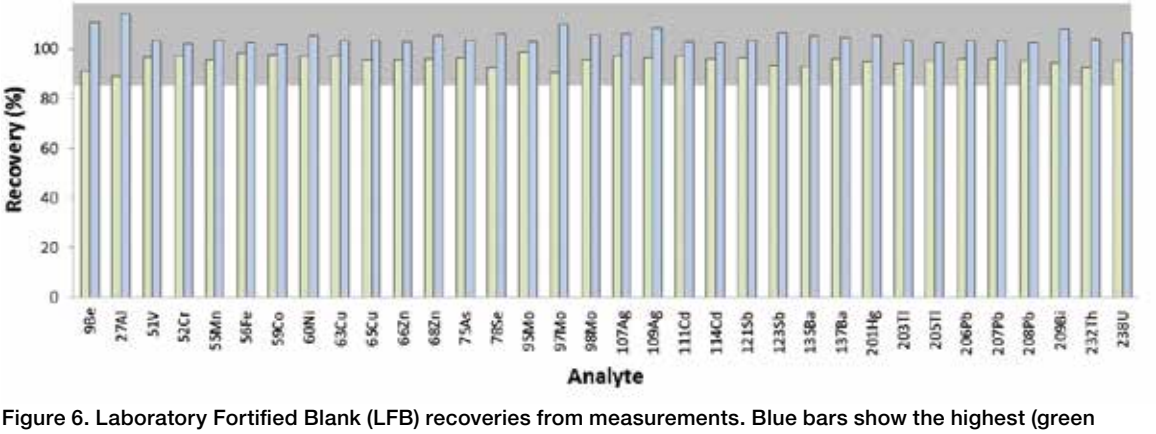


Figure 6. Laboratory Fortified Blank (LFB) recoveries from measurements. Blue bars show the highest (green lowest) recovery of the analyte measured during the 10 h run. The grey area represents the EPA 200.8 acceptance range (85-115%) for LFB recoveries.

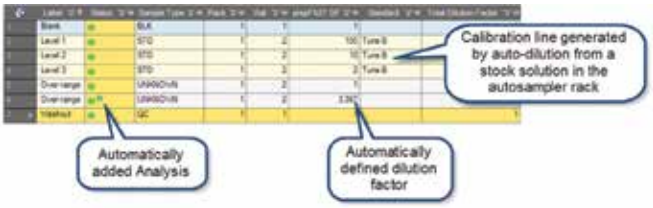


Figure 7. Screenshot of the intelligent auto-dilution process in Qtegra ISDS Software.

Conclusion

The Thermo Scientific iCAP RQ ICP-MS equipped with an ESI Autosampler and prep*FAST* Autodilution System was successfully validated for use with US EPA Method 200.8. With the robust iCAP RQ ICP-MS paired with an ESI prep*FAST* Autodilution system, it is possible to run the entire analysis (encompassing sample dilution, calibration and measurement) with minimal manual intervention. After optimizing the uptake and washout parameters, the high sensitivity and stability of the iCAP RQ ICP-MS readily achieved the goal of 52 EPA Method 200.8 analyses per hour.

Robustness

The iCAP RQ ICP-MS delivers reliable analysis of drinking water with minimal drift when equipped with the high matrix insert. For extra robust operation in the face of higher matrix samples, the system can be equipped with the robust plasma interface.

Productivity

The iCAP RQ ICP-MS in combination with the ESI prep*FAST* Autodilution System is the ideal system to measure environmental samples in a high throughput laboratory.

Simplicity

With the prescriptive and intelligent dilution capabilities provided by the system, manual sample preparation and data post-processing is minimized.

No Impact on Bench Space

The integrated dual valve assembly is mounted directly beneath the sample introduction system, minimizing sample pathways.

Meeting the requirements of water analysis regulations in Europe

The Water Framework Directive is a European Union directive. Its goal is to establish a framework for the protection of all water bodies within the EU area and it commits EU member states to achieving a “good status” for all ground and surface waters (including coastal waters).

Here John Quick, Principal Scientist from ALS Environmental, outlines the importance of the Water Framework Directive and how challenges faced by environmental testing labs are being overcome.

Q: What compounds should be monitored and what are the recommended methods of analysis?

John Quick (JQ): A daughter directive of the WFD – the Environmental Quality Standards Directive – establishes a list of 45 priority substances with set Environmental Quality Standard (EQS) concentration values. For surface waters, meeting “good chemical status” means that no concentrations of priority substances exceed the relevant EQS values. The priority substances list was based on risk assessments, it includes 4 metals (lead, cadmium, mercury and nickel) with all the rest being organic compounds covering a diverse range of chemical classes including pesticides, volatile organic compounds (VOCs), PAHs and industrial chemicals. Some of the EQS values specified in the directive are very small indeed and present a significant challenge to analytical chemists. The EQS for Cypermethrin in inland surface waters for example is 80pg/L (or 80 parts-per-quadrillion (ppq)) and in coastal waters it is an order of magnitude lower than this at 8pg/L (ppq). The WFD does not specify or recommend the methods of analysis to be used but another daughter directive – the QA/QC Directive - defines the minimum performance criteria that must be met. In a nutshell it states that the relative uncertainty (U%) must not be greater than 50% at the EQS level, that the LOQ (quantification limit) must not exceed 1/3 of the EQS value and that all methods must be validated and documented in accordance with the ISO 17025 standard. So long as these criteria are met, laboratories are free to choose their own methods of analysis.

Q: What are some of the biggest challenges faced by environmental testing labs?

JQ: The main analytical challenges surround an ever-expanding list of target analytes together with a need to report to ever lower limits of detection. The last decade has seen a growing requirement to analyse for an expanding list of emerging organic pollutants in environmental matrices such as endocrine disrupting hormones, pharmaceutical compounds, polyfluoroalkyl substances (PFAS), personal care products and so on. At the same time the required limits of detection have been driven significantly lower, in some cases by several orders of magnitude. A decade ago, our lowest limits of detection were in the low ng/L range (parts-per-trillion (ppt)), however we are now routinely reporting results in the low pg/L (ppq) range as in the case of Cypermethrin given above. We also operate in a highly competitive environment therefore we need to be able meet these technical challenges whilst seeking to minimise costs and maximise productivity.

Q: How do they overcome these challenges?

JQ: Access to highly sensitive and selective instrumentation is obviously a key factor in meeting these analytical challenges. We have invested heavily in GC and HPLC instruments which utilise both tandem mass spectrometry (MS/MS) and high-resolution mass spectrometry to achieve this and I strongly believe that having access to both of these powerful technologies means that we are able to deliver the best possible analytical methods for such a wide range of target analytes. Since we operate in a busy commercial environment it is also important that these instruments are both relatively easy to use and display good robustness when in routine operation. We are also seeking to maximise our efficiency by miniaturising and automating the sample preparation for many of our more routine methods and are investing in laboratory robotics to achieve this.

Q: How do labs safeguard themselves from future regulation changes?

JQ: I think "forewarned is forearmed" is the key here! Having a good working relationship and dialogue with your customers is important in being able to spot new analytical requirements before they hit, giving yourself valuable preparation time. I also believe it is important that laboratories look to "future proof" themselves as far as is possible when it comes to purchasing instrumentation and designing analytical methodologies.

John Quick, Principal Scientist
ALS Environmental

thermo scientific

APPLICATION NOTE 10702

An automated approach for the determination of gasoline range organics (GRO) in water by gas chromatography coupled with static headspace sampling

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Keywords
Gasoline range organics, GRO, water, valve and loop, headspace-gas chromatography, HS-GC, flame ionization detector, FID, TriPlus 500 HS, environment

Goal

The aim of this application note is to demonstrate the quantitative performance of the Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace (HS) Autosampler for the determination of gasoline range organics in water.

Introduction

Gasoline range organics (GRO) refer to hydrocarbons with a carbon range from C6 to C10 that have boiling points ranging from 60 °C to 170 °C. These chemicals are often present in the environment, especially in ground water and soil, mainly as a consequence of contamination incidents. The source of contamination can be human errors and accidents (such as oil spills) that occur when handling, storing, or transporting oil and oil products. If GRO are detected, the level of contamination needs to be determined by using quantitative analytical methods; therefore, this represents a routine application for environmental analysis laboratories. GRO are highly volatile compounds that can be easily extracted from the matrix without the need for time-consuming sample preparation. Therefore, the analytical technique of choice for this application is headspace sampling coupled to gas chromatography and mass spectrometry and/or flame ionization detection.

In this work, the headspace sampling technique coupled with gas chromatography-FID detection was employed to assess method sensitivity, precision, robustness, and linearity for quantitative assessment of GRO in water.

Experimental

In all experiments, a TriPlus 500 HS autosampler was directly interfaced (without the need for an external transfer line) to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with a Thermo Scientific™ Instant Connect split/splitless SSL Injector and a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID). Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS GC column, 30 m × 0.32 mm × 3.0 µm (P/N 26099-4840). Additional HS-GC-FID parameters are detailed in Table 1. The GC oven temperature program was optimized to reduce the analysis time and improve sample throughput; all peaks of interest elute in <13 minutes and the autosampler overlapping capability allows for long unattended sequences with automatic cycle time optimization.

Data acquisition, processing, and reporting

Data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2. Integrated instrument control ensures full automation from instrument set-up to raw data processing, reporting, and storage. Simplified e-workflows deliver effective data management ensuring ease of use, sample integrity, and traceability.

Standard and sample preparation

GRO standard mix at 1000 µg/mL was purchased from Restek (P/N 30095) and serially diluted using tap water to obtain seven stock solutions ranging from 6.25 µg/L to 10,000 µg/L (ppb). An amount of these standard stock solutions (5 mL) was transferred into a 10 mL crimp cap headspace vial (vials P/N 10CV, caps P/N 20-MCBC-ST3) and used to assess method linearity, sensitivity, recovery, and repeatability.

Sample preparation

Unleaded petroleum was diluted with reagent water to produce a sample stock solution at 5% and kept refrigerated at 4 °C. The sample stock was used to evaluate the matrix recovery and the quantitative accuracy and precision.

Table 1. HS-GC-FID operating conditions for GRO determination in water

TRACE 1310 GC Parameters	
Inlet Module and Mode:	SSL, split
Split Ratio:	20:1
Septum Purge Mode, Flow (mL/min):	Constant, 5
Carrier Gas, Carrier Mode, Pressure (kPa):	He, constant pressure, 150
Oven Temperature Program	
Temperature 1 (°C):	50
Hold Time (min):	1
Temperature 2 (°C):	220
Rate (°C/min):	15
Hold Time 2 (min):	5
FID	
Temperature (°C):	300
Air Flow (mL/min):	350
H_2 Flow (mL/min):	35
N_2 Flow (mL/min):	40
Acquisition Rate (Hz):	25

TriPlus 500 HS Autosampler Parameters	
Incubation Temp. (°C):	85
Incubation Time (min):	30
Vial Shaking:	Fast
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	200
Vial Pressure Equilibration Time (min):	1
Loop Size (mL):	1
Loop/Sample Path Temp. (°C):	105
Loop Filling Pressure (kPa):	150
Loop Equilibration Time (min):	1
Needle Purge Flow Level:	5
Injection Mode:	Standard
Injection Time (min):	1

Results and discussion

Method linearity

Linearity was evaluated by injecting seven calibration levels at 6.25, 12.5, 25, 50, 1000, 2500, and 10,000 µg/L (ppb). A list of target compounds is reported in Table 2. Each concentration level was prepared and analyzed in triplicate (n = 3). The calculated correlation coefficients (R^2) were 1.000 for all the investigated gasoline organics. Moreover, the residual values (measured as % RSD of average response factors) were <6.5%, confirming an excellent linearity (Figure 1).

Detection limit and accuracy assessment (recovery)

The method detection limit is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.[2] According to the Wisconsin method[3] for GRO determination, the required limit of quantitation (LOQ) is 100 µg/L (ppb) or less for water samples and the method blank should not exceed a concentration of 50 µg/L (ppb). The method detection limit (MDL) was assessed analyzing n = 7 blank tap water samples (5 mL) and n = 7 tap water samples spiked at the concentration of 12.5 µg/L (ppb). MDL and LOQ were then calculated applying Equations 1 and 2, respectively.

The recovery was calculated using Equation 3 and was in the range 80% to 120%, with an average value of 105%. MDL, LOQ, and percent recovery results for the spiked samples are reported in Table 2. None of the investigated compounds could be detected in the tap water samples as shown in Figure 2.

(Equation 1)

$$MDL = t_{(n-1,1-\alpha=0.99)} * S$$

Where:
t = Student's *t*-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with *n*-1 degrees of freedom, for
n = 7 injections: *t* = 3.143
S = standard deviation of the replicate analysis

(Equation 2)

$$LOQ = 10 * S$$

Where:
S = standard deviation of the replicate analysis

(Equation 3)

$$\text{Average } \%R = (C_{ave}/C_{sp}) * 100\%$$

Where:
C_{ave} = average concentration of the spiked samples
C_{sp} = initial spike concentration

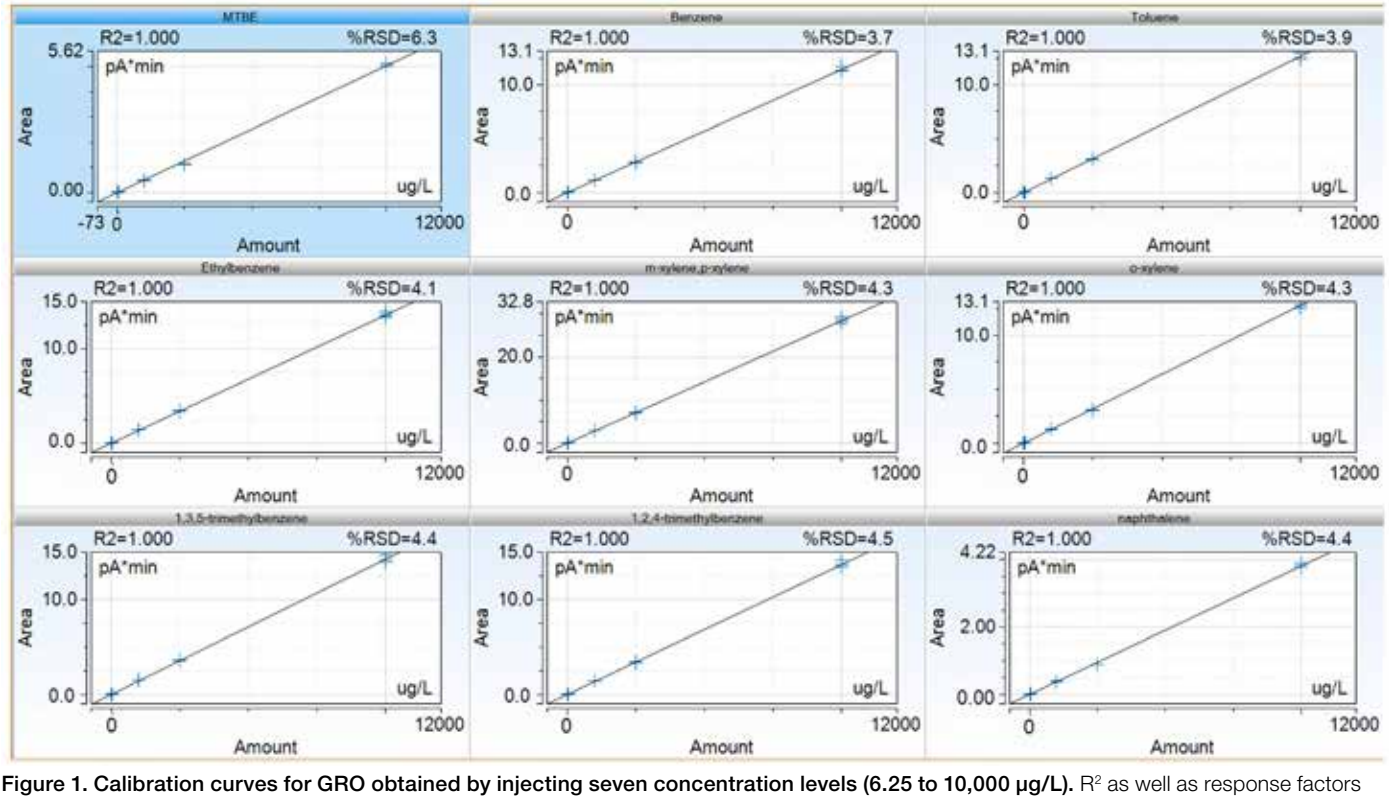


Figure 1. Calibration curves for GRO obtained by injecting seven concentration levels (6.25 to 10,000 µg/L). R^2 as well as response factors relative standard deviations (% RSD) are shown. Each calibration level was prepared and analyzed in triplicate (n = 3).

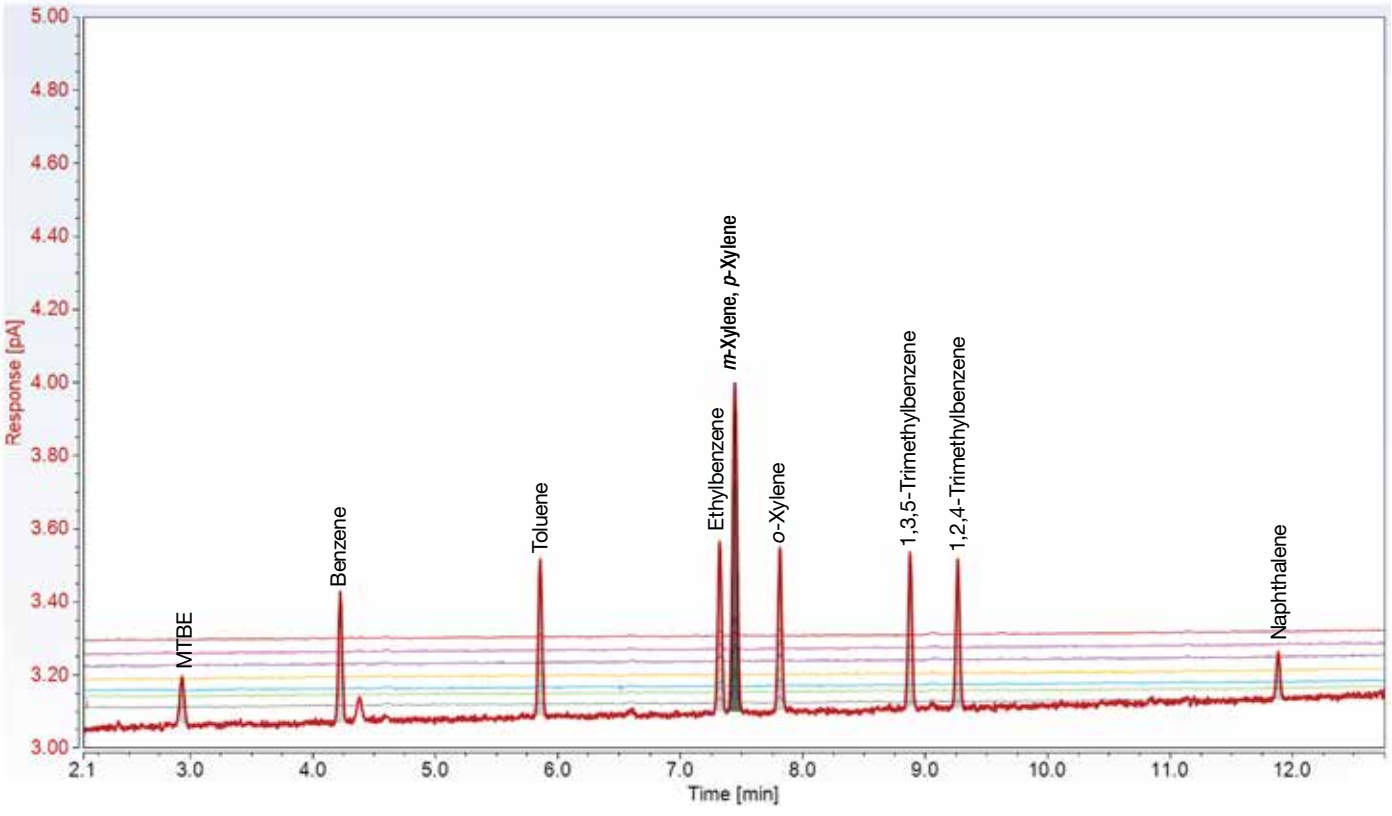


Figure 2. Comparison between chromatograms obtained analyzing n = 7 real tap water samples (unspiked) and a tap water sample spiked at 12.5 µg/L (ppb) (red trace). None of the investigated gasoline organics could be detected in the unspiked tap water samples.

Table 2. Calculated MDL, LOQ, and % recovery for n = 7 tap water samples spiked at a concentration level of 12.5 µg/L (ppb)

Gasoline Range Organics	Spiked Conc. (µg/L)	Average Measured Conc. (µg/L, n = 7)	Calculated MDL (µg/L)	Calculated LOQ (µg/L)	Average Recovery (%, n = 7)
Methyl *tert*-butyl ether (MTBE)	12.5	11.5	1.4	4.4	92
Benzene	12.5	12.8	1.2	3.9	103
Toluene	12.5	13.7	1.7	5.5	110
Ethylbenzene	12.5	12.8	1.3	4.0	102
m-Xylene, *p*-Xylene	12.5	12.8	0.8	2.7	103
o-Xylene	12.5	12.4	0.8	2.6	100
1,3,5-Trimethylbenzene	12.5	14.4	1.7	5.5	115
1,2,4-Trimethylbenzene	12.5	13.3	1.7	5.3	107
Naphthalene	12.5	14.6	2.2	7.1	117
Average		**13.1**	**1.4**	**4.6**	**105**

To assess the method accuracy (%) in tap water samples containing raw gasoline matrix, 30 µL of the sample stock solution (prepared as described in the sample preparation section) were diluted into two flasks previously filled with 30 mL of tap water and fortified with the standard solution at a concentration of 1000 µg/L (ppb) and 10,000 µg/L (ppb). A blank matrix solution was prepared by adding 30 µL of sample stock solution to 30 mL tap water. Then, 5 mL of each fortified solution and the blank matrix were transferred into 10 mL headspace vials (n = 5) and analyzed to assess the recovery. The average recoveries (%) for the spiked matrix samples were calculated using Equation 3 and confirmed to be within 80–120% of the spiked levels with an average value of 96.5% (Table 3). Chromeleon CDS matrix correction feature allowed for automated subtraction of the background leading to a precise quantitation of the spiked samples.

Precision

System repeatability was assessed using n = 10 consecutive injections of tap water samples spiked at a concentration of 50 µg/L (ppb) and n = 10 tap water samples spiked with the 5% raw gasoline solution. Peak area %RSDs obtained for both assessments are reported in Table 4. Excellent repeatability was obtained for both standard and matrix spiked samples with an average %RSD of 0.91 and 1.1, respectively.

Table 4. Peak area %RSDs obtained from n = 10 consecutive injections of tap water spiked with the standard solution at 50 µg/L (ppb) and n = 10 consecutive injections of tap water spiked with diluted raw gasoline. Average peak area %RSDs are 0.91 and 1.1 respectively.

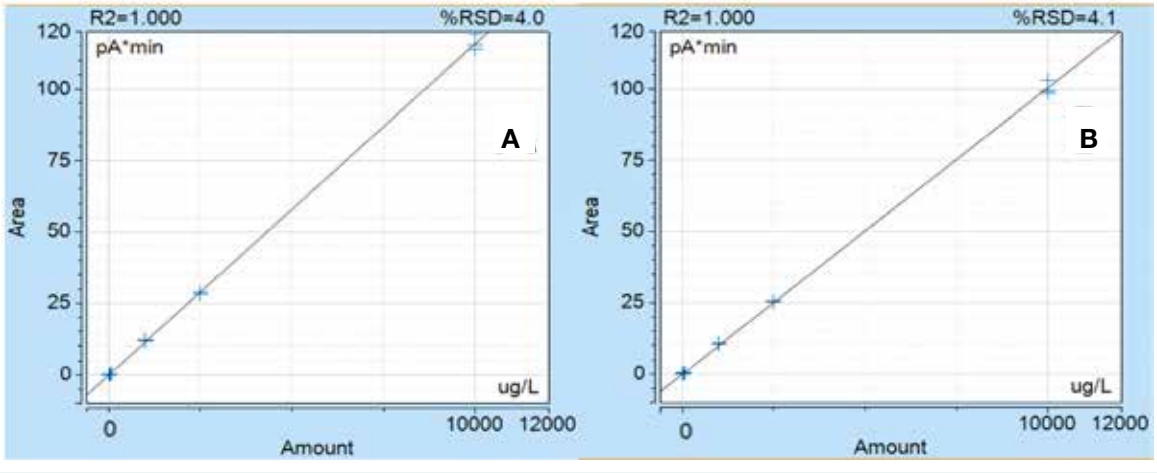
Gasoline Range Organics	Peak area %RSD	
	Tap Water Spiked with Stock Solution (n = 10)	Tap Water Spiked with Raw Gasoline (n = 10)
Methyl *tert*-butyl ether (MTBE)	1.0	1.0
Benzene	0.93	1.2
Toluene	0.87	1.1
Ethylbenzene	0.78	0.8
m-Xylene, *p*-Xylene	0.85	1.5
o-Xylene	0.92	1.2
1,3,5-Trimethylbenzene	0.98	1.2
1,2,4-Trimethylbenzene	0.99	1.1
Naphthalene	0.82	1.2
Average	**0.91**	**1.1**

Table 3. Calculated recoveries (%) for n = 5 tap water samples spiked with diluted raw gasoline and fortified with standard solution at a concentration of 1000 and 10,000 µg/L (ppb). Average concentrations are calculated subtracting the raw gasoline matrix.

Gasoline Range Organics	Average Blank Matrix Conc. (µg/L, n = 5)	Spiked Conc. 1 (µg/L)	Average Measured Conc. (µg/L, n = 5)	Average Recovery (%, n = 5)	Spiked Conc. 2 (µg/L)	Average Measured Conc. (µg/mL, n = 5)	Average Recovery (%, n = 5)
Methyl *tert*-butyl ether (MTBE)	7	1000	1,130	113	10,000	10,300	103
Benzene	4	1000	890	89	10,000	9,300	93
Toluene	142	1000	990	99	10,000	9,300	93
Ethylbenzene	25	1000	890	89	10,000	9,400	94
m-Xylene, *p*-Xylene	54	1000	900	90	10,000	9,300	93
o-Xylene	54	1000	920	92	10,000	9,300	93
1,3,5-Trimethylbenzene	8	1000	910	91	10,000	9,400	94
1,2,4-Trimethylbenzene	31	1000	920	92	10,000	9,200	92
Naphthalene	7	1000	1,160	116	10,000	10,500	105
Average			**970**	**97**		**9,600**	**96**

Quantitation of GRO in real water samples

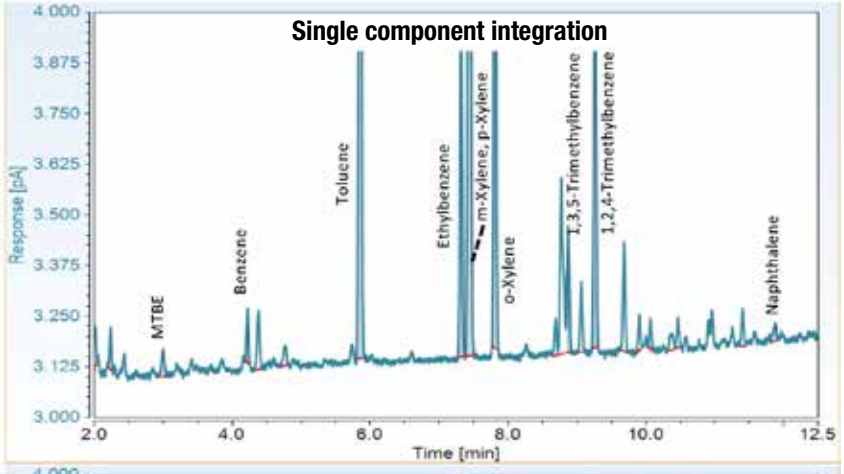
Tap water samples (5 mL, n = 10) were spiked with 1 µL of raw gasoline solution (5%) and analyzed. According to Wisconsin and EPA method 8015 C,[4] *GRO quantitation is based on a direct comparison of the total area within a defined retention time window to the total peak areas of the gasoline component standard.* Therefore, the calibration curves previously plotted using the single component peak integration were calculated integrating the total peak area and used to quantitate the spiked water samples. The total area was obtained integrating all the chromatographic peaks within the retention time window ranged from MTBE (RT = 2.92 min) to naphthalene (RT = 11.96 min) according to the Wisconsin method and from 2-methylpentane (RT = 2.62 min) to 1,2,4-trimethylbenzene (RT = 9.25 min) according to EPA 8015 C method. The *"baseline to baseline"* integration did not include the solvent peak. Calculated correlation coefficient (R^2) were 1.000 and the residual values (measured as % RSD of average response factors) were ~4% for both retention time windows confirming an excellent linearity. MDL, LOQ, and recovery were calculated for the total peak area calibration curves applying Equations 1, 2, and 3. Calibration curves and calculated MDL, LOQ, and percent recovery (total area integration applied) are shown in Figure 3. As an example, a chromatogram of a tap water sample (5 mL) spiked with raw gasoline solution (5%) (single component and EPA 8015 C total area integration) as well as the quantitation results obtained for the analyzed samples (single components and total area quantitation) are reported in Figure 4. A series of blank water vials (n = 5) was run after completing the sample sequence. No compound carry-over was detected in the blanks as demonstrated in Figure 5.



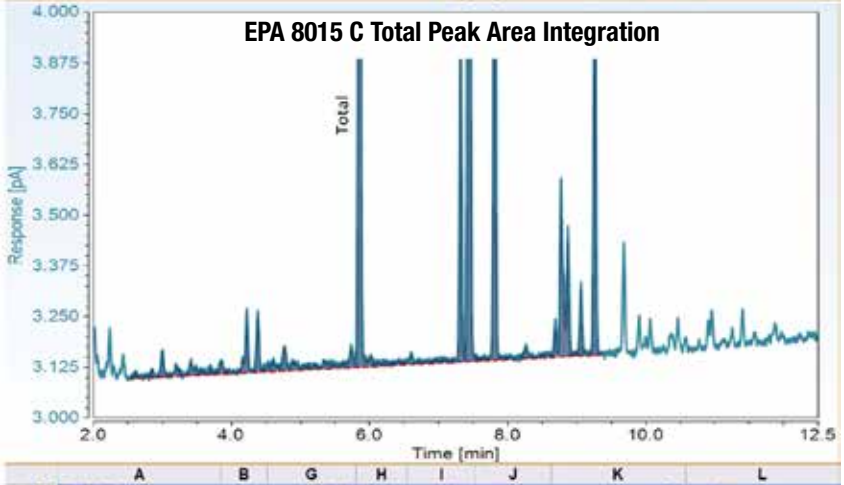
C

Gasoline Range Organics	Spiked Conc. (µg/L)	Average Measured Conc. (µg/L, n = 7)	Calculated MDL (µg/L)	Calculated LOQ (µg/L)	Average Recovery (%, n = 7)
Total Area Integration (Wisconsin method)					
Total	12.5	11.4	1.9	6.1	91
Total Area Integration (EPA 8015 C method)					
Total	12.5	13.0	2.2	7.0	105

Figure 3. Calibration curves were obtained integrating the total area within the gasoline range at each calibration level for Wisconsin (A) and EPA 8015 C (B). R^2, response factor relative standard deviations (% RSD) as well as calculated MDL, LOQ, and percent recovery (C) are shown.



A



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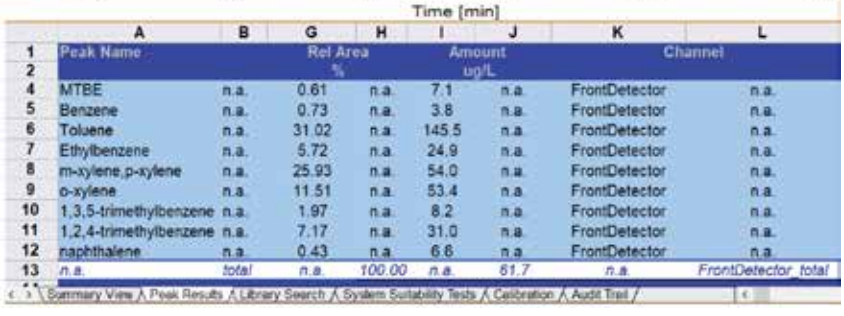

	A	B	G	H	I	J	K	L
1	Peak Name		Rel Area		Amount		Channel	
2			%		ug/L			
4	MTBE	n.a.	0.61	n.a.	7.1	n.a.	FrontDetector	n.a.
5	Benzene	n.a.	0.73	n.a.	3.8	n.a.	FrontDetector	n.a.
6	Toluene	n.a.	31.02	n.a.	145.5	n.a.	FrontDetector	n.a.
7	Ethylbenzene	n.a.	5.72	n.a.	24.9	n.a.	FrontDetector	n.a.
8	m-xylene,p-xylene	n.a.	25.93	n.a.	54.0	n.a.	FrontDetector	n.a.
9	o-xylene	n.a.	11.51	n.a.	53.4	n.a.	FrontDetector	n.a.
10	1,3,5-trimethylbenzene	n.a.	1.97	n.a.	8.2	n.a.	FrontDetector	n.a.
11	1,2,4-trimethylbenzene	n.a.	7.17	n.a.	31.0	n.a.	FrontDetector	n.a.
12	naphthalene	n.a.	0.43	n.a.	6.6	n.a.	FrontDetector	n.a.
13	*n.a.*	*total*	*n.a.*		*100.00*	*n.a.*	*61.7*	*n.a.*	*FrontDetector_total*

Summary View / Peak Results / Library Search / System Suitability Tests / Calibration / Audit Trail /

C

Gasoline Range Organics	Average Measured Conc. (µg/L, n = 10)	Average Measured Conc. (µg/L, n = 10)	
	Single Component Integration	Total Area Integration (Wisconsin)	Total Peak Area Integration (EPA 8015 C)
Methyl *tert*-butyl ether (MTBE)	7.1	53.3	56.0
Benzene	3.7		
Toluene	141.2		
Ethylbenzene	24.8		
m-Xylene, *p*-Xylene	53.1		
o-Xylene	53.7		
1,3,5-Trimethylbenzene	8.0		
1,2,4-Trimethylbenzene	31.1		

D

Figure 4. Example of tap water sample (5 mL) spiked with raw gasoline solution (5%) chromatogram applying single component integration (A) and total area integration (EPA 8015 C integration window), (B). Chromeleon "Peak Results" view (C) allows the display of the peak results for both integration types. Average quantitative results for n = 10 tap water samples spiked with raw gasoline and integrated using single components and total area are reported in the table (D).

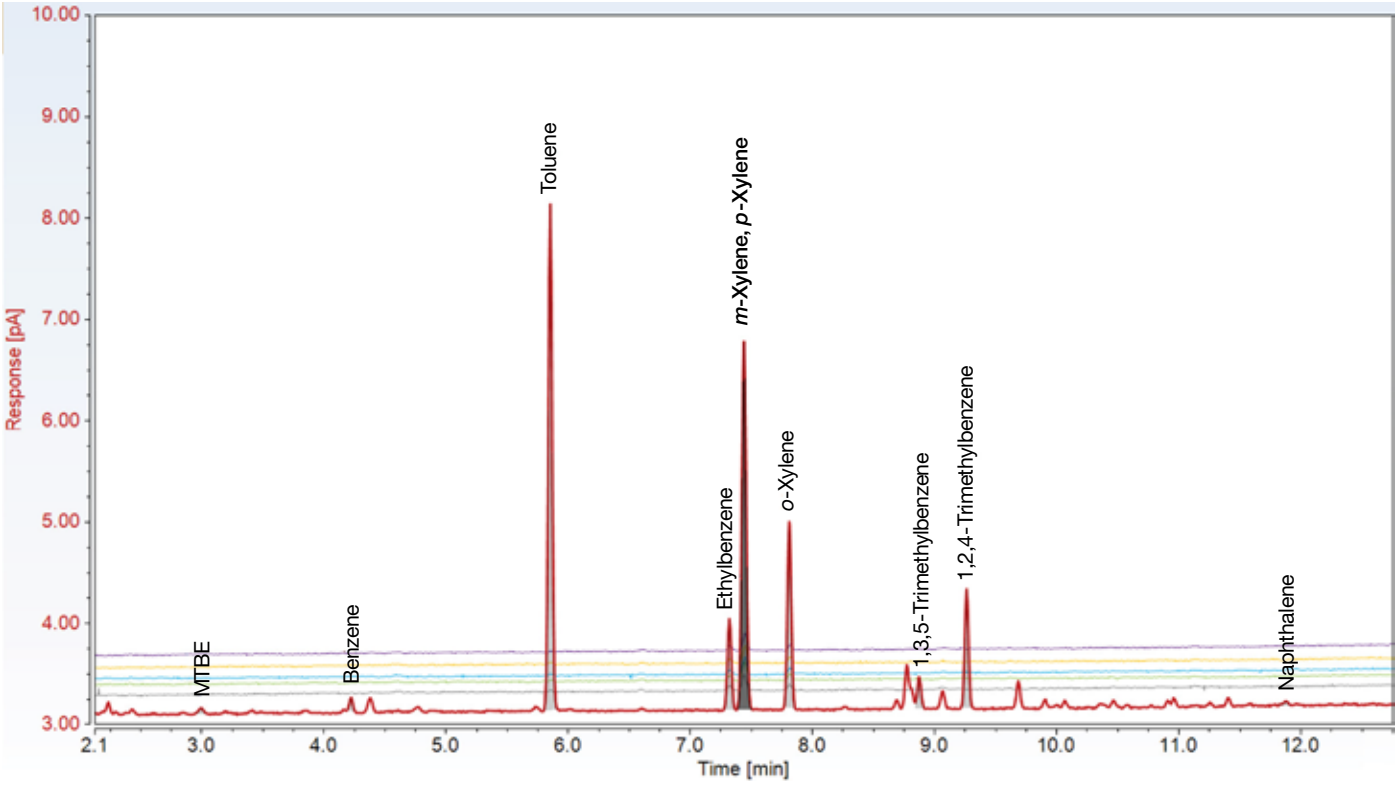

Figure 5. Comparison between chromatograms obtained analyzing n = 5 blank water vials after completing the sample sequence and a sample spiked with 1 µL of raw gasoline solution (5%) (red trace). None of the investigated gasoline organics or any residual matrix components could be detected in the blank water.

Conclusions

The results presented here demonstrate the suitability of the TriPlus 500 HS autosampler in combination with the Trace 1310 GC-FID for GRO analysis in environmental samples.

- Excellent linearity with correlation coefficient $R^2 = 1.000$ was obtained for all analytes. The Instant Connect Flame Ionization Detector (FID) allows sensitive detection of organic compounds as demonstrated by the calculated MDL and LOQ (average MDL = 1.4 µg/L (ppb) and average LOQ = 4.6 µg/L (ppb)).
- The advanced Quick Spin Shaking (QSS) feature of vials and direct column connection to the valve manifold ensure efficient analyte extraction. In the experiments performed here, the average compound recovery for matrix spiked samples was >96%.
- The low bleed and superior inertness of the TraceGOLD column allowed for highly reliable results. The high column efficiency allowed for a fast GC oven ramp supporting short analysis time (all analytes elute in <13 min) and high sample throughput to easily meet the needs of routine laboratories. Moreover, up to 240 sample vials can be accommodated into the trays for unattended 24-hour operations.
- The pneumatic control and the sample path inertness of the TriPlus 500 HS autosampler ensure reliable and reproducible analyte injection and transfer. Average peak area RSDs (n = 10 consecutive injections) were 0.91% for tap water samples spiked with the standard solution at 50 µg/L (ppb) and 1.1% for tap water spiked with diluted raw gasoline.
- The efficient purging of the pneumatic circuit of the TriPlus 500 HS autosampler eliminated potential for carry-over; no matrix components or gasoline organics were detected in the blank vials after a sequence of real samples contaminated with GRO chemicals.
- Quantitation of spiked samples is simplified with the Chromeleon CDS advanced reprocessing features allowing for easy single component and total peak area integration and compound quantitation.

Overall, the data shows that the TriPlus 500 gas chromatography static headspace autosampler provides a reliable analytical tool allowing environmental laboratories to produce consistent results with outstanding analytical performance for GRO quantitative analysis in water samples.

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thermo scientific

APPLICATION NOTE 44417

Direct analysis of trace elements in estuarine waters using triple quadrupole ICP-MS

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Keywords

Argon gas dilution, Contamination, Estuarine waters, Interference removal, Matrix effects

Goal

To show accurate quantification of toxic elements in a heavy sample matrix like estuarine waters using argon gas dilution.

Introduction

An estuary is an ecosystem, which is characterized by both marine (e.g. tides, or erosion through waves) and riverine processes (e.g. freshwater and sediment inputs). At the interface between fresh- and sea water domains, estuarine waters are often referred to as brackish waters with salinities between 1 and up to 35 (corresponding to total dissolved solids between 0.1 - ≤ 3.5%). Coastal zones including estuaries are historically populated by humans, hosting major cities and industrial activities. Estuaries are the focal points of aquatic contaminant transport from the continent to the sea, facing contamination from industrial, agricultural and urban sources, especially over the past decades. Important examples for estuaries are e.g. Puget Sound in the northwestern US, Rio de la Plata in South America, or the Thames Estuary in the UK. In this study, the focus is on the Gironde Estuary in southwest France, a major fluvial-estuarine system in Europe. The main objective of this study is to track and control historical and ongoing multi-metal contamination of the Gironde Estuary waters. Clear evidence for contamination (mainly Cd) has been observed in seafood (oysters) from the estuary mouth and the nearby Marennes-Oléron Bay, Europe´s major oyster production area[1].

However, the analysis of samples containing high salt loads imposes special challenges when using ICP-MS. Salts may crystalize during the process of nebulization or deposit on surfaces of the interface region, leading to blockage of nebulizers and cone orifices. This may lead to severe reduction of signals and cause drift problems. With respect to spectral interferences, most commonly chlorine based polyatomics, such as those interfering on vanadium, chromium and arsenic, need to be removed. Additionally, strong interferences can be observed on copper, based on the presence of sodium and magnesium in estuarine and sea waters.

In order to overcome the impact of the sample matrix, samples can be diluted using clean diluents, but especially for the analysis of elements at trace or ultra-trace levels, dilution always induces the risk of contamination or overdilution, leading to final concentrations in the measured solution being lower than instrumental detection limits (IDL). Another appealing alternative is the use of argon gas to dilute the sample before it enters the plasma. Although this also leads to a significant reduction in achievable instrument sensitivity, method detection limits (taking into account all steps in sample preparation) can be less compromised as compared to liquid dilution.

Method

Sample preparation

Estuarine water samples were collected in the high salinity range of the Gironde Estuary i.e. relatively close to the estuary mouth, ~100 km downstream from Bordeaux, France. Sampling has been performed onboard the research vessel Thalia (Ifremer) over a cycle of 30 hours, implying that the water masses sampled have variable salinity due to the strong ebb-flood cycle in this meso-/macrotidal estuary. Salinities in the samples ranged from S = 30.2 to 31.8. To avoid potential matrix effects caused by different salinity, all samples were adjusted to a salinity of S=30. The star in Figure 1 shows the sampling site in the Gironde Estuary mouth, being part of a larger sampling campaign along the entire estuarine salinity gradient (data not shown).

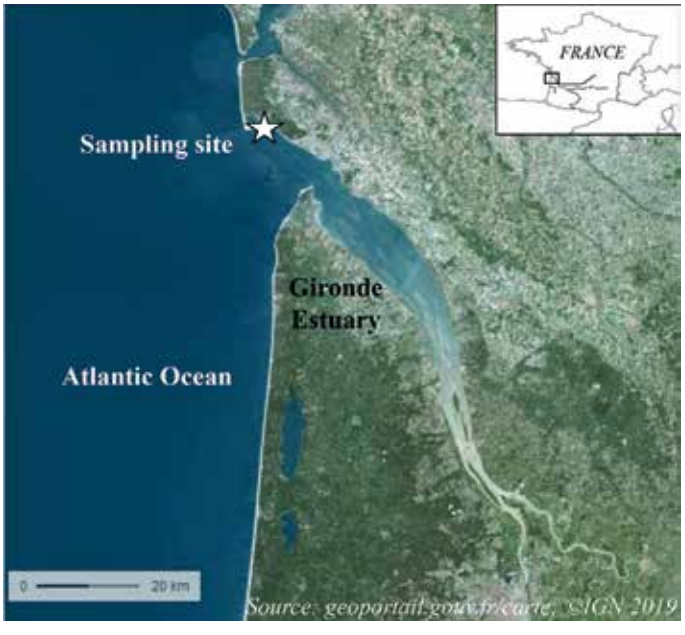

Figure 1. Sampling location along the Gironde Estuary.

Due to the extremely low concentrations in seawater commonly observed for some of the analytes (especially Pb, but also Cd), careful control over potential sources of contamination and clean laboratory conditions are key to successful analysis. The labware was acid-cleaned (soaking 3 days in 10% HNO_3 Normapure®, VWR-BDH Chemicals), thoroughly rinsed with MilliQ® water (Merck), dried under a laminar flow hood in a clean lab (over-pressurized, filtered, air-conditioned atmosphere), then sealed in double plastic bags until use. All samples were filtered onboard immediately after sampling using 0.2 µm membrane filters (MINISART® NML, Sartorius), acidified (1/1000, HNO_3 Suprapur®, Merck), and stored in the dark at 4 °C pending analysis.

Instrument configuration

A Thermo Scientific™ iCAP™ TQ ICP-MS in combination with an SC-4DX Autosampler (Elemental Scientific, Omaha, NE) was used for analysis. The instrument was operated using Argon Gas Dilution (AGD) allowing direct analysis of estuarine waters without any prior dilution. Tuning of the system was accomplished using the autotune routines provided with the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ Software. Typical operating conditions are summarized in Table 1. The selection of analytes, appropriate analysis mode (single quad vs. triple quad, choice of reactive gas) was accomplished using the Reaction Finder method development assistant. For the selected elements, this resulted in only arsenic being acquired in a triple quadrupole mode using oxygen as a reactive gas, whereas for all other analytes, the use of helium and kinetic energy discrimination was recommended. The mass selection in the first quadrupole was controlled using intelligent Mass Selection (iMS) in all cases.

Table 1. Instrument configuration.

Parameter	Value	
Nebulizer	MicroMist Quartz nebulizer 0.4 mL·min^{-1}, pumped at 40 rpm	
Spray chamber	Quartz cyclonic spray chamber cooled at 2.7 °C	
Injector	2.5 mm i.d., Quartz	
Interface	High Matrix (3.5 mm) insert, Ni cones	
RF power	1550 W	
Nebulizer gas flow	0.73 L·min^{-1}	
Additional gas flow (AGD)	97 %	
QCell settings	**SQ-KED**	**TQ-O_2**
Gas flow	100% He, 4.2 mL·min^{-1}	100% O_2, 0.3 mL·min^{-1}
CR bias	-21 V	- 6.4 V
Q3 bias	-18 V	-12 V
Scan settings	0.1 s dwell time per analyte, 10 sweeps, 3 main runs	

General analytical condition

For calibration and quality control, a certified reference material was used (CASS 6, Nearshore Seawater Certified Reference Material for Trace Metals and other Constituents, National Research Council Canada). Matrix matched calibration curves were generated by addition of increasing concentrations of the elements investigated in this study directly into aliquots of the CASS-6 CRM. Table 2 gives an overview on the added concentrations for the different analytes. As the calibration was matrix matched no internal standard was used in the analysis. Again, all solutions were adjusted to a salinity of S=30.

Following 8-9 unknown samples, CASS 6 was repeatedly analyzed by standard addition in order to (i) check the accuracy of the method and (ii) monitor potential sensitivity drift.

Table 2. Added concentrations for calibration curves per element.

	Cu	Zn	As	Cd	Pb
Zero STD	0	0	0	0	0
Standard 1 [µg·kg^{-1}]	0.5	1.3	1.0	0.02	0.01
Standard 2 [µg·kg^{-1}]	1.0	2.6	2.0	0.04	0.02
Standard 3 [µg·kg^{-1}]	1.5	3.9	3.0	0.06	0.03
Standard 4 [µg·kg^{-1}]	2.0	5.2	4.0	0.08	0.04
Standard 5 [µg·kg^{-1}]	2.5	6.5	5.0	0.10	0.05

Results

The results obtained are summarized in Table 3. As can be seen, quantitative recoveries are obtained for all elements under study in the CASS-6 CRM. The CRM was analyzed 4 times throughout the analysis and demonstrated low relative standard deviations despite the extremely low concentrations of some of the elements. For arsenic (the only element measured in both single and triple quadrupole modes), there is no significant difference in the results considering the uncertainty information in the certificate of the CASS 6 reference material. In this sample matrix, the predominant interference on ^{75}As are chlorine -and calcium-based species, such as $^{40}Ar^{35}Cl^+$, $^{40}Ca^{35}Cl^+$ or $^{40}Ca(OH)_2H^+$. Due to their polyatomic nature, these interferences can be efficiently removed by KED alone. However, it is worth noticing that the triple quadrupole based mode using oxygen offered a much higher detection sensitivity (more than double in comparison to KED) and significantly lower detection limits (more than 5 times lower). Other interferences, such as doubly charged ions of the Rare Earth Elements, might affect the results for elements such as arsenic or selenium, and can only be removed using triple quadrupole technology. However, in this study they were not found to be causing any bias to the results.

The results of this study were compared to an earlier study using a different analytical technique, i.e. a submersible voltammetry system validated for measuring estuarine samples[2]. The comparison shows that results are very similar for arsenic, cadmium and lead. Results for copper and zinc deviated slightly from earlier results probably due to their higher affinity for forming complexes with organic molecules present in sea water, which are not detected by in-situ voltammetry.

Table 3. Results obtained for the measurement of CASS 6 CRM and 18 samples.

Element	Cu	Zn	As		Cd	Pb
Mode	SQ-KED	SQ-KED	SQ-KED	TQ-O2	SQ-KED	SQ-KED
Result CASS 6 CRM [µg·kg^{-1}] (N=4)	0.57 ± 0.012	1.89 ± 0.23	1.04 ± 0.11	1.09 ± 0.08	0.027 ± 0.004	0.013 ± 0.002
Certified value [µg·kg^{-1}]	0.530 ± 0.032	1.27 ± 0.18	1.04 ± 0.10		0.0217 ± 0.0018	0.0106 ± 0.0040
Concentration range in samples [µg·kg^{-1}]	0.31-0.56	0.41-2.34	1.32-1.88		0.017-0.058	0.023-0.042

Conclusion

The direct analysis of estuarine waters without prior dilution is possible using AGD on the iCAP TQ ICP-MS. The results obtained for the CASS-6 CRM indicate accurate and precise quantification is possible at very low concentration levels. The results obtained for the samples collected in the high salinity range of the Gironde Estuary show that historical metal contamination in the estuarine waters persists, although at lower levels (especially for elements such as Cd or Zn) as compared to earlier studies conducted in the 1990`ies[3]. This observation fits with the continuous decrease of Cd concentrations determined in wild oysters from the Gironde Estuary mouth[4]. Comparing the data to the results of an earlier study, it is clear that both, ICP-MS and voltammetry methods, provide similar results at trace and ultra-trace levels. Slight differences in results may occur due to metal species/complexes not detected in voltammetry. If submersible voltammetry systems allow for in-situ measurements of a number of trace metals, ICP-MS is a time-efficient alternative and allows the analysis of a wider range of elements in one aspiration of the sample due to its inherent multi-elemental capability. Future work on multi-element analysis in seawater should include an even wider range of elements, including emerging metal contaminants.

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Acknowledgement

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APPLICATION NOTE 10490

Discovery of emerging disinfection by-products in water using gas chromatography coupled with Orbitrap-based mass spectrometry

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Keywords

Iodinated disinfection by-products, water, accurate mass, high resolution, Q Exactive GC

Introduction

The disinfection of drinking water is required in order to protect consumers from potential waterborne infectious and parasitic pathogens. Water is commonly treated by adding chemical disinfectants, such as free chlorine, chloramines, chlorine dioxide, and ozone. However, although very effective in removing disease-causing microorganisms, these disinfectants can react with naturally occurring materials in the water and can form disinfection by-products (DBPs) which can be harmful to human health. In particular, compounds containing an iodo-group, i.e., iodinated DBPs (iodo-DBPs), may pose a greater health risk for the population exposed to them than their brominated and chlorinated analogues.[1] In recent years, several chemical classes of low molecular weight iodo-DBPs have been reported; however, many more may be still present in the unknown fraction (~50%) of halogenated material formed during disinfection treatments.[2] Therefore, complete characterization of iodo-DBPs present in DBP mixtures is crucial to further investigate their occurrence in disinfected waters and potential toxicity effects.

The identification of emerging iodinated DBPs in water is difficult due to the complexity of this matrix and the low concentrations of these compounds. For this, analytical techniques with high resolving power, high mass accuracy and sensitivity are required. In this work, a novel gas chromatography (GC), coupled with high-resolution accurate mass Orbitrap mass spectrometer (the

Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer), has been used for iodo-DBPs detection and accurate mass identification in chlorinated and chloraminated water samples.

Experimental

Sample Preparation

The formation of DBPs is mainly related to the type of the disinfection treatment applied, and the nature of the water source in terms of natural organic matter (NOM) characteristics, as well as the bromide and iodide content. In order to study the formation of iodo-DBPs in iodine-containing waters, lab-scale chlorination and chloramination reactions were performed.

The tested water was a Milli-Q® water solution containing NOM from the Nordic reservoir (NL) (Vallsjøen, Skarnes, Norway), which is a reference material from the International Humic Substances Society (IHSS), fortified with bromide (500 ppb, added as KBr) and iodide (50 ppb, added as KI). Following disinfection reactions with chorine and monochloramine, the water samples were extracted onto XAD resins, and analytes retained were eluted with ethyl acetate. After drying and concentration of these extracts, they were directly injected into the Q Exactive GC system for analysis of iodo-DBPs.

Details about the procedures followed to perform the disinfection reactions and DBP analysis can be found elsewhere.[3]

A procedural blank, i.e., untreated water concentrated in the same manner as the treated samples, was used to investigate whether the compounds detected and identified were generated during disinfection treatments or were artifacts generated during the sample preparation treatments.

GC-MS conditions

Compound separation and detection was achieved using a Thermo Scientific™ TRACE™ 1310 GC system coupled with a Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler. The analytical column used was a Thermo Scientific™ TG-5MS, 60 m × 0.25 mm ID × 0.25 µm film thickness (P/N: 26096-1540). Additional details of instrument parameters are shown (Tables 1 and 2).

Table 1. GC Temperature program.

TRACE 1310 GC System Parameters	
Injection Volume (µL):	1.0
Liner:	Single taper, wool (P/N 453A0924-UI)
Inlet (°C):	280
Inlet Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1
Temperature 2 (°C):	325
Rate (°C/min):	15
Hold Time (min):	10

Table 2. Mass spectrometer parameters.

Q Exactive GC Mass Spectrometer Parameters	
Transfer Line (°C):	280
Ionization Type:	EI & CI (methane)
Ion Source (°C):	230 (EI), 185 (CI)
Electron Energy (eV):	70
Acquisition Mode:	Full-scan
Mass Range (Da):	50 - 650
Resolving Power (FWHM at *m/z* 200):	60,000
Lockmass, Column Bleed (*m/z*):	207.03235

Data processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software that allowed peak detection with spectral deconvolution and tentative compound identification against a commercial spectral library (NIST). In order to reduce chemical interferences from the matrix, a mass window of ± 2 ppm was always used to enable generation of highly selective extracted ion chromatograms. Semi-quantitative information (peak area) was also obtained and a sample comparison was conducted in order to find chemicals that are only present in the treated samples analyzed.

Results and discussion

The DBP mixture concentrates obtained from the lab-scale chlorination and chloramination reactions were analyzed in full scan mode. An example of chromatographic separation is shown in Figure 1 for untreated-control and chlorinated samples.

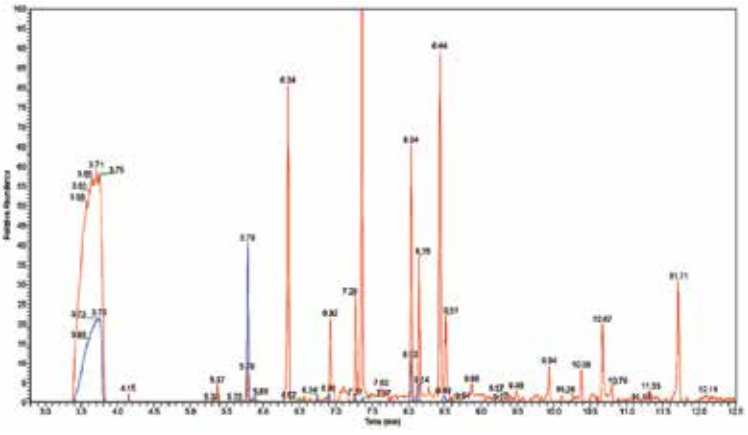


Figure 1. Overlayed extracted ion chromatograms (*m/z* 126.90392, iodine) of Milli-Q water spiked with natural organic matter (NL NOM) subjected to chlorination (red) and control of untreated water (blue) showing an increase in both the number and intensity of iodine-containing peaks in the chlorinated water as compared to the control.

Compound discovery workflow

The workflow used for the detection and molecular structure characterization of iodo-DBPs is schematically represented in Figure 2. Data acquired in full scan using electron ionization (EI) was processed in TraceFinder for peak detection and spectral deconvolution followed by compound identification using a library (NIST) search and high-resolution filtering (HRF) of the candidate compounds. The deconvolution software uses a HRF score for the library searches. For each compound with a library match, the HRF represents the relative number of explainable ions in the measured spectra as compared to the proposed elemental composition of the best (based on the forward search index SI value) library match.[4] Consequently, the confidence in compound identification is dramatically increased as the analyst does not only rely on a library matching score (such as the forward SI).

Data processing was simultaneously performed for all DBP mixtures generated (i.e., untreated NL NOM, chlorinated NL NOM and chloraminated NL NOM). A large number of peaks were detected subsequent to deconvolution (e.g., >2,500 peaks were found in the chloraminated NL NOM extract using a total ion current (TIC) intensity threshold of 500,000 and a signal-to-noise (S/N) threshold of 10:1). Having a high number of component peaks is clearly beneficial for comprehensive characterization of a sample. However, it is also essential for users to quickly isolate the peaks of interest, either within a sample or between sample groups. To facilitate this, TraceFinder has a variety of filters that can be used to isolate particular features in the data. In this example, an exact mass filter was used to isolate only the compounds containing iodine (exact mass *m/z* 126.90392). This reduced the total list of iodine containing chemicals detected to only 15 main peaks in the aforementioned example, i.e., chloraminated NL NOM extract.

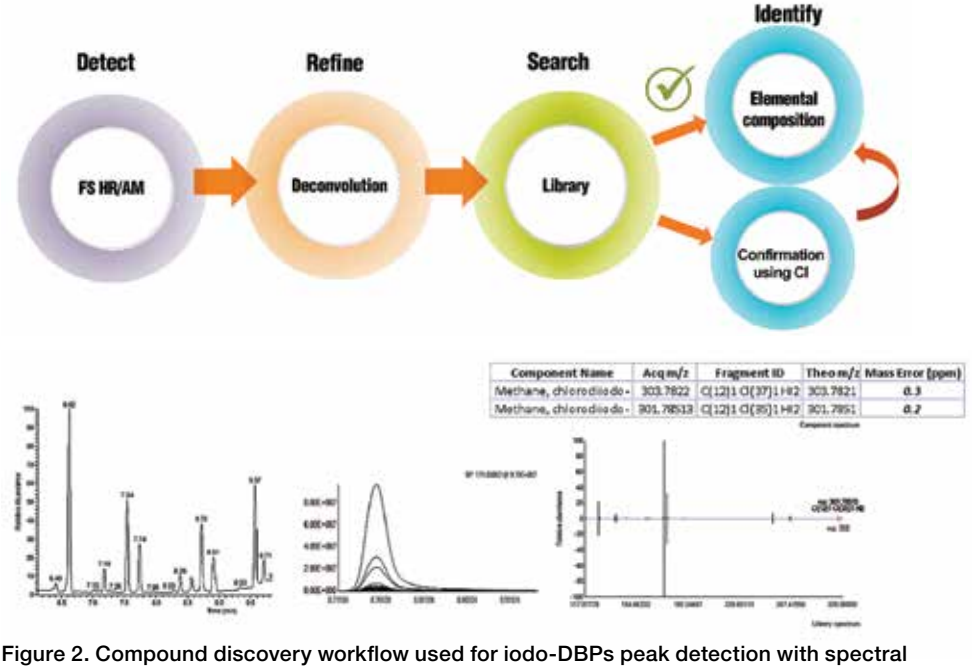


Figure 2. Compound discovery workflow used for iodo-DBPs peak detection with spectral deconvolution and tentative compound identification.

An example of peak deconvolution in the TraceFinder's browser is shown in Figure 3 for chlorodiiodomethane. The samples of interest (a) were deconvoluted and a list of peaks was generated (b). Tentative compound identification was made by searching the NIST library, taking into account the forward search index (SI). In addition, an HRF score was used to determine the percentage of the mass fragments in the acquired spectrum that can be explained by the chemical formula of the molecular ion proposed from the library match, in this case $CHCII_2$ for chlorodiiodomethane. This resulted in a combined total score indicating the quality of match between this library hit and the deconvoluted measured spectrum. This functionality makes this software a very powerful and unique tool that can be used for compound identification and confirmation.

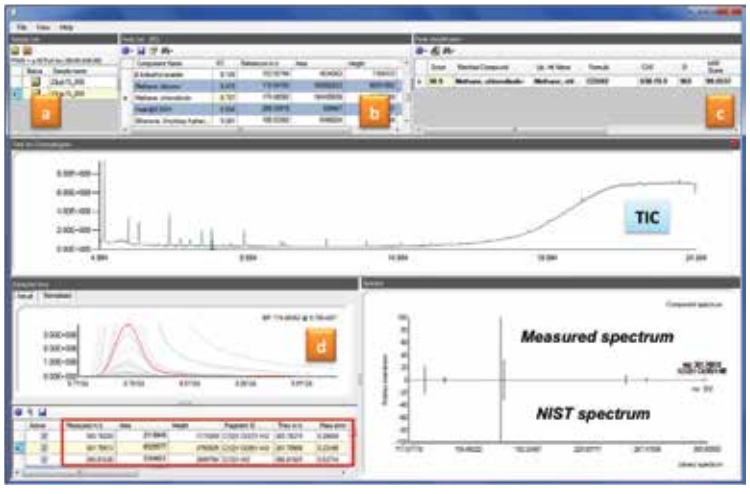


Figure 3. Deconvolution browser showing chlorodiiodomethane identification based on library (NIST) match search index, SI 963), fragment rationalization with an HRF> 99% and mass accuracies of measured fragments (e.g., molecular ion m/z 301.78513 ppm = 0.23). Samples processed (a), peaks detected (b), identified chemicals (c), and deconvoluted mass spectra for chlorodiiodomethane (d) with the measured and theoretical ions including mass errors are indicated.

Identification of Iodo-DBPs with no library match

However, many emerging chemical contaminants do not have a match in NIST (or similar MS libraries) and in this case a different approach has to be used to determine their identity (elemental composition and chemical structure). This is where obtaining high mass accuracy becomes critical as only with appropriate mass spectral data is it possible to clearly determine the elemental composition of an unknown chemical.

In this work, the EI mass spectra of the compounds detected in the treated water samples did not provide a sufficient match in the NIST library, and were interrogated using a pre-determined set of chemical elements (C-50, H-50, Br-5, Cl-10, I-10, O-10, and N-10). The molecular ion of the target compound was confirmed using positive chemical ionization (PCI) with methane. In addition, authentic standards were analyzed to confirm the identities using the retention time, EI mass spectral match, and mass accuracy of the measured ions. An example of unknown identification for compounds with no spectral match in the NIST library is shown in Figure 5 for iodoacetaldehyde.

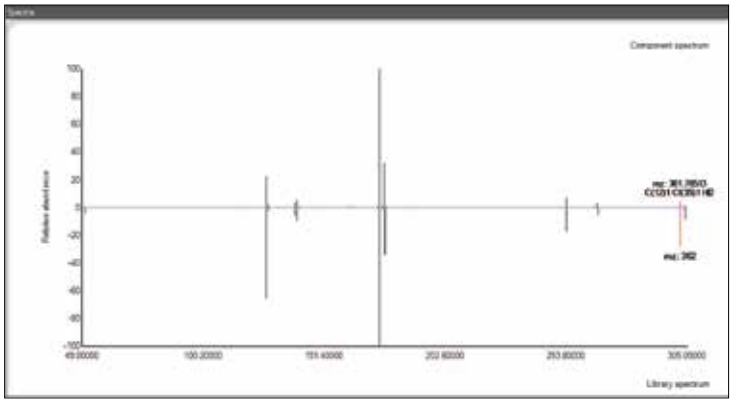


Figure 4. Ion mass spectrum, corresponding accurate masses (ppm) and elemental composition of chlorodiiodomethane (RT= 8.77 min) a) in the chloraminated NL NOM extract and b) MS library match. Data acquired in EI at 60,000 resolution (FWHM, at *m/z* 200). Annotated are the acquired fragment ions that can be explained from $CHCII_2$ proposed by NIST. Automatic elemental composition calculation is determined for each ion in the spectra in addition to exact mass calculations and mass difference (ppm error).

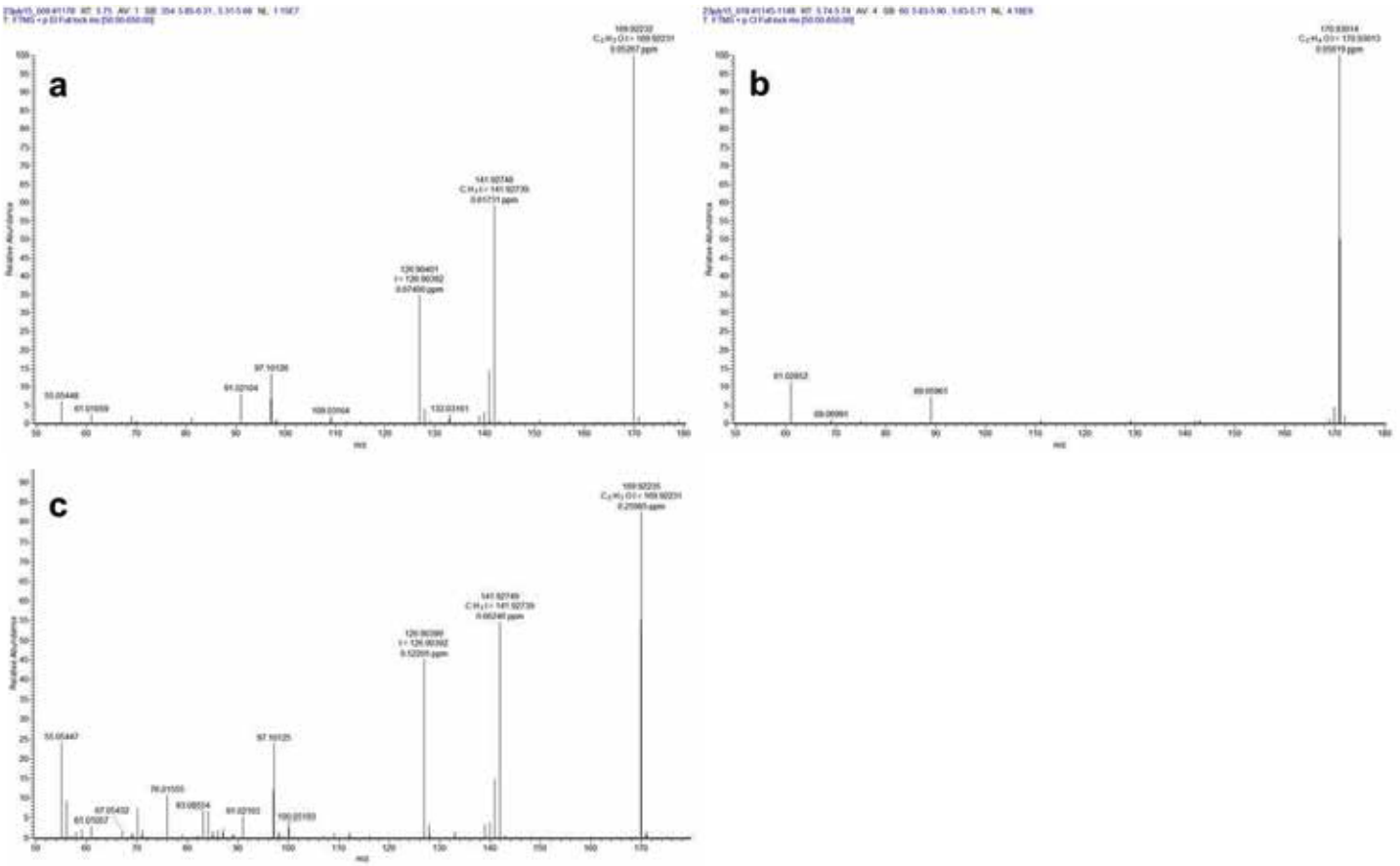


Figure 5. Confirmation of iodoacetaldehyde identification with authentic solvent standard (a) and NL treated samples (c) based on RT and mass accuracy measurements. Positive chemical ionization (PCI) mass spectrum (b) confirms mass of molecular ion $[M+H]^+$ with 0.06 ppm mass accuracy.

Sample comparison and fold-change of Iodo-DBPs

As an additional approach to identifying peaks of interest, TraceFinder software also allows for sample grouping and facilitates the analysis and data visualization of fold changes of the analytes detected. Detected peaks in all the samples were retention time aligned and the peak areas automatically compared, resulting in the generation of a heat map (Figure 6). This semi-quantitative approach allows the researcher to easily visualize and report the levels of detected chemicals.

Increased levels of iodo-DBPs were observed following chloramination (NH_2Cl) reactions, in agreement with what was previously reported.[5] Following the identification workflow described above, a total of eight different iodo-DBPs were confidently identified in the extracts analyzed. Chemical structures were proposed for all compounds after applying the workflow described in the previous section. Experimental and theoretical masses of molecular ions from both EI and PCI with methane, the mass difference (Δ ppm), the assigned elemental compositions for each diagnostic ion, and the proposed chemical structure for the identified DBPs are shown in Table 3.

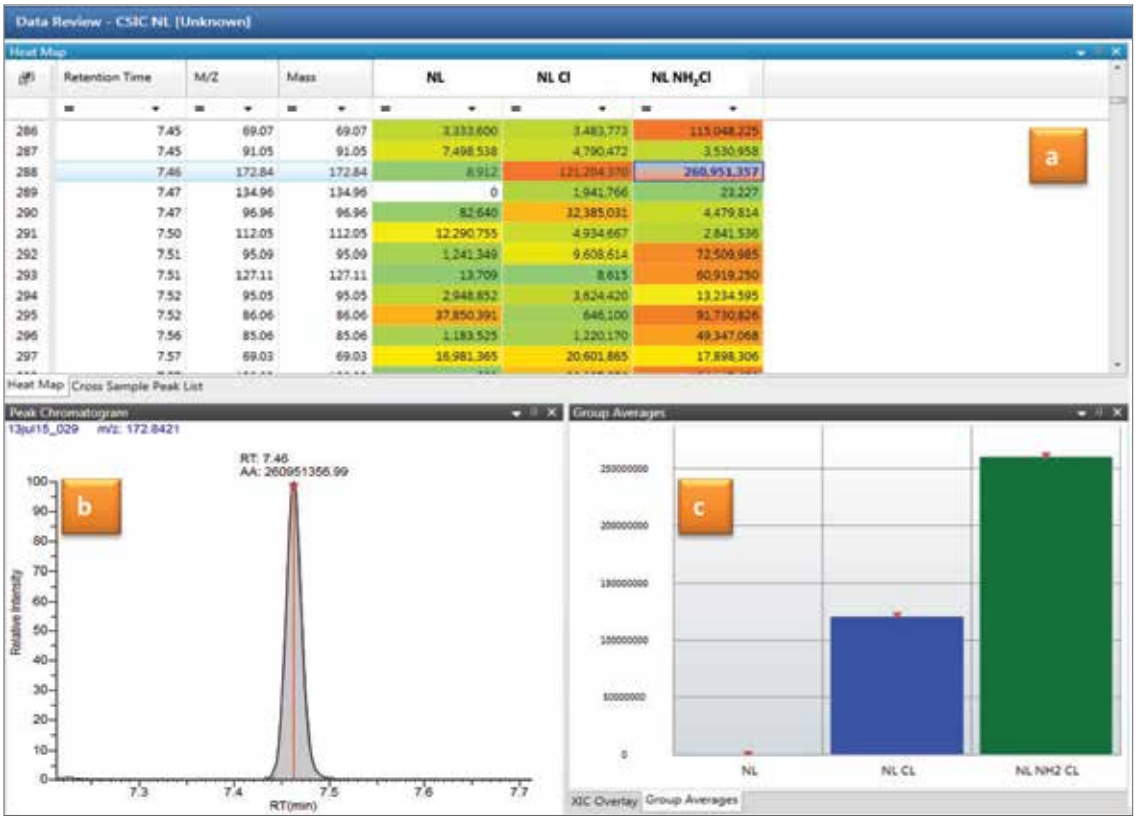
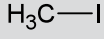
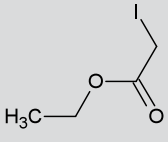
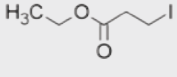
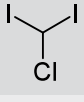
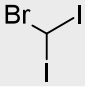


Figure 6. TraceFinder browser showing the heat map with the peak areas of detected peaks (a), and as an example, the increased concentration of a compound eluting at RT = 7.46 min, the corresponding extracted peak chromatogram (b), and the abundance of this chemical in the samples analyzed (c).

Table 3. Iodo-DBPs identified and confirmed in disinfected NL NOM waters.

RT (min)	Identity	Elemental Composition	Chemical Structure	Theoretical *m/z* (EI)	Measured *m/z* (EI)	Δ (ppm)	Theoretical *m/z* $[M+H]^+$	Measured *m/z* $[M+H]^+$	Δ (ppm)
3.71	Iodomethane	CH_3I		141.92739	141.92745	0.4	142.93522	142.93522	0.0
5.36	Chloroiodomethane	CH_2ClI		175.88842	175.88839	0.2	176.89625	176.89620	0.3
5.76	Iodoacetaldehyde	C_2H_3IO		169.92231	169.92234	0.2	170.93013	170.93014	0.06
7.36	Diiodomethane	CH_2I_2		267.82404	267.82424	0.8	268.83186	268.83192	0.2
8.03	Ethyl iodoacetate	$C_4H_7IO_2$		213.94852	213.94840	0.6	214.95635	214.95627	0.4
8.14	ethyl β-iodopropionate	$C_5H_9IO_2$		n.d.	n.d.	—	228.97200	228.97198	0.07
8.77	Chlorodiiodomethane	$CHClI_2$		301.78507	301.78509	0.1	301.78507	301.78511	0.1
9.85	Bromodiiodomethane	$CHBrI_2$		345.73455	345.73459	0.1	345.73455	345.73446	0.3

Sample comparisons revealed that significantly higher levels of DBPs were observed in the chloraminated samples compared to the chlorinated extracts. Peak areas (XIC of *m/z* 126.90392) in the chloraminated extract were 8 to 66-fold higher as compared to the chlorinated extract, and up to 145 in the case of diiodomethane (Figure 7).

Conclusions

- This work has shown the successful application of the Q Exactive GC system for the characterization of iodo-DBPs in disinfected water extracts.
- A large number of peaks were detected in the samples analyzed and an exact mass filter in TraceFinder was used to isolate only the compounds containing iodine. Higher concentrations of iodo-DBP were found in the samples exposed to chloramination compared to chlorination treatments.
- The EI data obtained can be used for candidate compound identification against existing commercial libraries. Importantly, as often the chemicals detected are not included in such libraries, the consistent sub-ppm mass accuracy measurements will unambiguously determine the elemental composition and subsequent structural elucidation of unknown chemicals.
- Moreover, softer ionization such as positive chemical ionization with methane can be used to confirm the elemental composition of the molecular ion of a chemical.
- The Q Exactive GC mass spectrometer and the compound discovery and identification workflow described here allow for rapid detection and confident identification of unknown DBPs in disinfected water, enabling researchers to reliably and timely report the identities of the unknown chemicals.

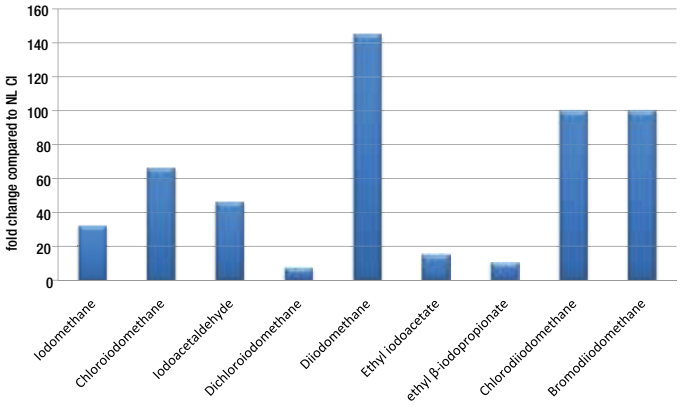


Figure 7. Fold increase of iodo-DBPs detected and identified in chloraminated DBP mixture concentrates as compared to chlorinated ones.

Acknowledgements

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APPLICATION NOTE 10644

Overcoming analytical challenges for polybrominated diphenyl ethers (PBDEs) analysis in environmental samples using gas chromatography – Orbitrap mass spectrometry

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Keywords

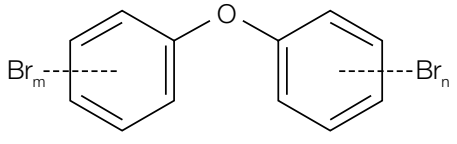
Polybrominated diphenyl ethers, PBDE, high-resolution GC-MS, accurate mass, quantification, GC Orbitrap, environmental, sediment, filter dust, sludge, air

Goal

To demonstrate the quantitative performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS mass spectrometer for the analysis of polybrominated diphenyl ethers (PBDEs) in environmental samples.

Introduction

Polybrominated diphenyl ethers (Figure 1) are a group of organobromine chemicals that inhibit or suppress combustion in organic material. They have been widely used since the 1970s as flame retardants in a broad range of commercial and household products including textiles, building materials, electronics, furnishings, motor vehicles, and plastics.[1]



where m + n = 1 to 10

Figure 1. Structure of polybrominated diphenyl ethers (PBDEs)

Most PBDEs resist degradation, persist and bioaccumulate in both the environment and food chains, and can be transported through air and water over long distances. They have been identified, in some cases far from their place of use, in a wide range of samples including air, water, sediment, fish, birds, marine mammals, and humans.[2] Many PBDEs are toxic, with links to cancer and endocrine disruption.[3] As a consequence, the use of certain PBDEs (including penta-, tetra-, and deca-BDE) have been prohibited in many countries and are currently listed in the Stockholm Convention inventory of persistent organic pollutants.[4]

Due to their chemico-physical properties, gas chromatography (GC) is the standard analytical technique used to analyze PBDEs, with detection using either an electron capture detector (ECD), or a mass spectrometer (MS). However, there are many analytical challenges to consider when using gas chromatography-mass spectrometry (GC-MS) for the analysis of PBDEs. The active nature of high molecular mass PBDEs (e.g. BDE-209), the large number of compounds, and the potential chromatographic interferences from matrix (e.g. chromatographic separation of BDE-49 and BDE-71 can be challenging in complex environmental samples).

This work demonstrates the applicability of high-resolution, accurate-mass GC-Orbitrap technology for the targeted analysis of 27 PBDE congeners in environmental samples with variable complexity using a sensitive, fast, robust method. This approach takes into account the selectivity, sensitivity, linearity, reproducibility of the results, method robustness, and analysis time.

Experimental conditions

Sample preparation

The following environmental samples were provided by the Dioxins Laboratory, IDAEA-CSIC, Barcelona, Spain: three sediment samples (including two samples previously used in an inter-laboratory study, and one sample previously used in a QA/QC study), three sludge samples (from a waste water treatment plant), three filter dust samples (previously used in a QA/QC study), and one air sample (previously used in an inter-laboratory study).

Samples (2 g), were Soxhlet extracted with toluene for 24 hours, followed by a basic alumina purification stage (6 g), activated overnight at 300 °C, and elution with 50 mL n-hexane/DCM (80:20). The extracts were then blown to dryness and reconstituted with 20 µL nonane prior to GC-MS analysis. A mass-labelled (^{13}C) PBDE surrogate standard was added prior to extraction and a mass-labelled (^{13}C) PBDE recovery standard was added prior to injection, as illustrated in the PBDE analytical workflow (Figure 2).

Instrument and method setup

An Exactive GC Orbitrap GC-MS mass spectrometer coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph was used in all experiments.

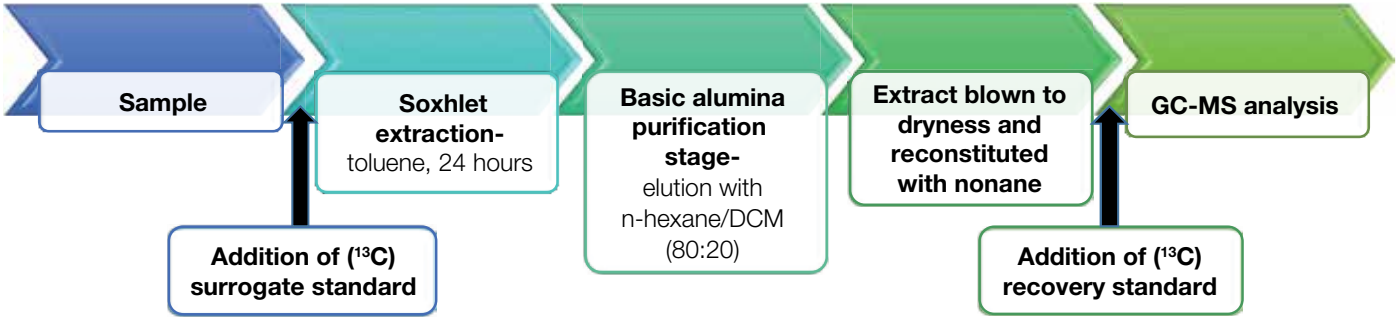


Figure 2. PBDE analytical workflow, including sample extraction, extract purification, and concentration stages required prior to GC-MS analysis

Liquid sample injections were performed with a Thermo Scientific™ TriPlus™ RSH™ autosampler, using the Thermo Scientific™ Instant Connect Programmed Temperature Vaporizing (PTV) injector for the TRACE 1300 GC system. Compound separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-PBDE 15 m × 0.25 mm I.D. × 0.10 µm film capillary column (P/N 26061-0350). The mass spectrometer was tuned and calibrated in <1.5 min using FC43 (CAS 311-89-7) to achieve mass accuracy of <0.5 ppm. The system was operated in electron ionization mode (EI) using full-scan, and 60,000 mass resolution (full width at half maxima, measured at *m/z* 200). Additional details of instrument parameters are shown in Table 1 and Table 2.

Table 1. GC and injector conditions

TRACE 1310 GC system parameters

Injection volume:	1.0 µL
Liner:	PTV baffled liner (Siltek™) (P/N: 453T2120)
Inlet:	40 °C
Carrier gas, (mL/min):	He, 1.5 mL/min
Inlet module and mode:	PTV, Large Volume mode
Column:	TraceGOLD TG-PBDE 15 m × 0.25 mm I.D. × 0.10 µm film capillary column (P/N 26061-0350)
Transfer delay:	0.2 min
Injection time:	0.1 min

PTV Parameters:	Rate (°C/s)	Temperature (°C)	Time (min)	Flow (mL/min)
Injection	—	40	0.10	—
Transfer	2.5	330	5.00	—
Cleaning	14.5	330	5.00	50

Oven Temperature Program:	RT (min)	Rate (°C/min)	Target Temperature (°C)	Hold Time (min)
Initial	0	—	100	2.00
Final	2.00	30	340	3.00
Run time	13.00	—	—	—

Table 2. Mass spectrometer conditions

Exactive GC mass spectrometer parameters

Transfer line temperature:	300 °C
Ionization type:	EI
Ion source:	250 °C
Electron energy:	35 eV
Acquisition modes:	Targeted SIM/full-scan
Mass range:	68–1000 Da
Isolation window:	25 Da
Mass resolution:	60,000 FWHM at *m/z* 200

Calibration standards (BDE-CSV-G), containing 27 native PBDE congeners at five concentration levels (Appendix A), and 16 (^{13}C labelled) PBDE internal standards (Appendix B), were acquired from Wellington Laboratories, Inc. (Ontario, Canada).

Targeted screening experiments were developed for the PBDE congeners considered. The targeted-SIM inclusion list, start and end times, and PBDEs included within each entry are given in Appendix C.

Data processing

Data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.2. Chromeleon CDS allows the analyst to build acquisition, processing, and reporting methods for high-throughput analysis, with easy data reviewing and data reporting.

Results and discussion

The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for the quantification of PBDEs to increase sample throughput and laboratory productivity. Various analytical parameters, including chromatographic resolution, instrument sensitivity, and linearity, were assessed and the results of these experiments are described below.

Chromatography

Good chromatographic separation, in <11 minutes, was obtained using the GC conditions described in Table 1. Extracted ion chromatograms (EICs) for 27 native PBDE congeners in a mixed solvent standard are shown in Figure 3a, with the excellent chromatographic resolution of the critical pair (BDE-49 and BDE-71) highlighted (Figure 3b).

Quantification

The quantitative performance of the Exactive GC Orbitrap GC-MS system was tested for all 27 PBDEs. System sensitivity, linearity, and peak area repeatability were evaluated. Additionally, mass accuracy of the target compounds was assessed across the concentration ranges. Linearity was assessed using five calibration levels (1 to 400 pg on column for mono- to penta-BDEs, 2 to 800 pg on column for hexa- to octa-BDEs, and 5 to 2000 pg on column for nona- to deca-BDEs).

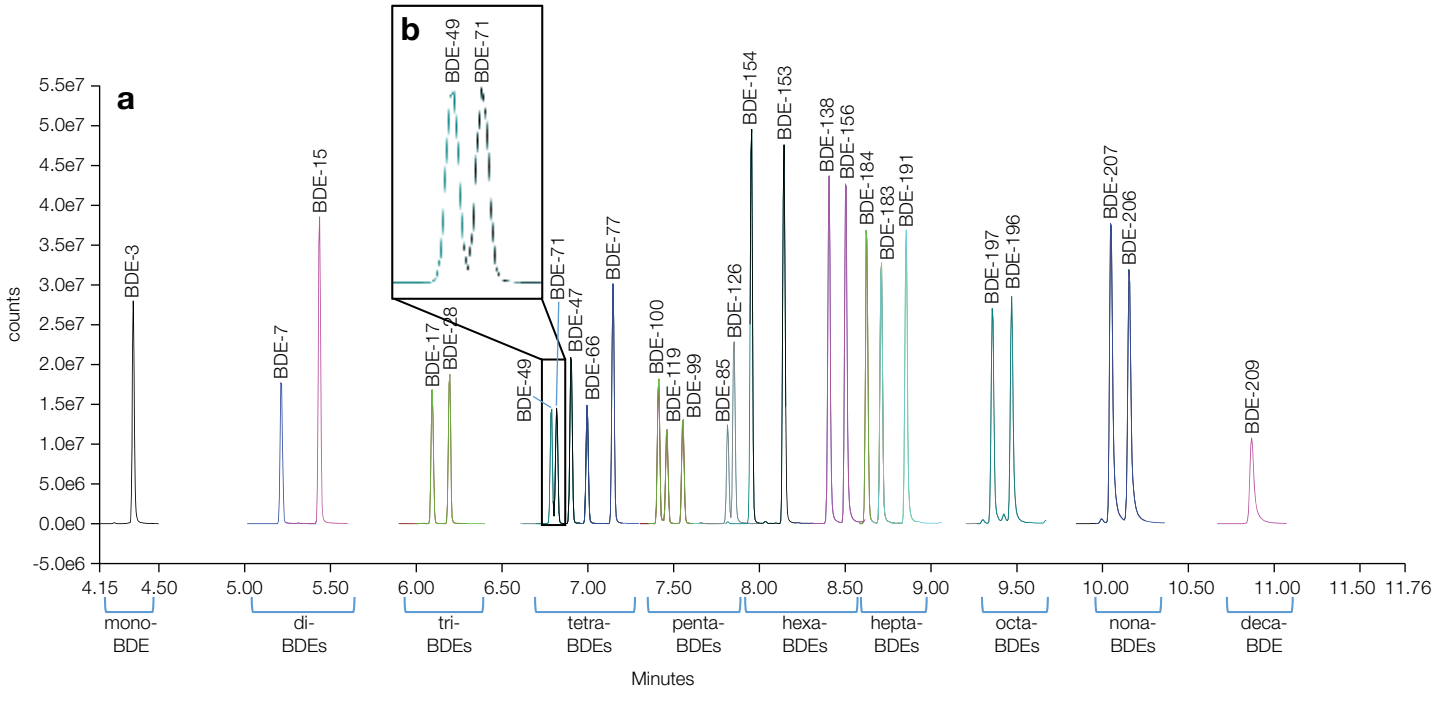


Figure 3. (a) Overlaid extracted ion chromatograms (EICs ±5 ppm extraction window) for the 27 native PBDE congeners in a solvent standard at 400 pg on column for mono- to penta-BDEs, 800 pg on column for hexa- to octa-BDEs, and 2000 pg on column for nona- to deca-BDEs and (b) separation of critical pair (BDE-49 and BDE-71)

Data was acquired using targeted-SIM, with compound detection, and identification based on retention time (±0.1 min window), accurate mass (±5 ppm window), and ion ratio of quantification vs. confirming ion (±15% window). Details of the calibration range, retention times, quantification and confirming ions, and ion ratio average values and acceptable ranges are shown in Appendix D.

Sensitivity

All PBDEs were detected in the lowest calibration standard, 1.0 ng/mL for mono- to penta-BDEs, 2.0 ng/mL for hexa- to octa-BDEs, and 5 ng/mL for nona- to deca-BDEs.

Estimation of instrument detection limit (IDL)

System sensitivity, defined as instrument detection limit (IDL) were determined experimentally for each compound by performing n=14 replicate injections of the lowest serially diluted solvent standard (with PBDE concentrations ranging from 50 to 100 fg on column). Calculations of IDLs were made using a one-tailed student *t*-test at the 99% confidence interval for the corresponding degrees of freedom and taking into account the concentration on column for each PBDE congener and absolute peak area %RSD (Figure 4).

Mass accuracy

Maintaining mass accuracy and spectral fidelity is critical for correct compound identification in complex environmental samples. Figure 5 illustrates the mass accuracy and the isotopic pattern match achieved for BDE-209 with mass accuracy of <2 ppm consistently achieved for each ion in the isotopic cluster.

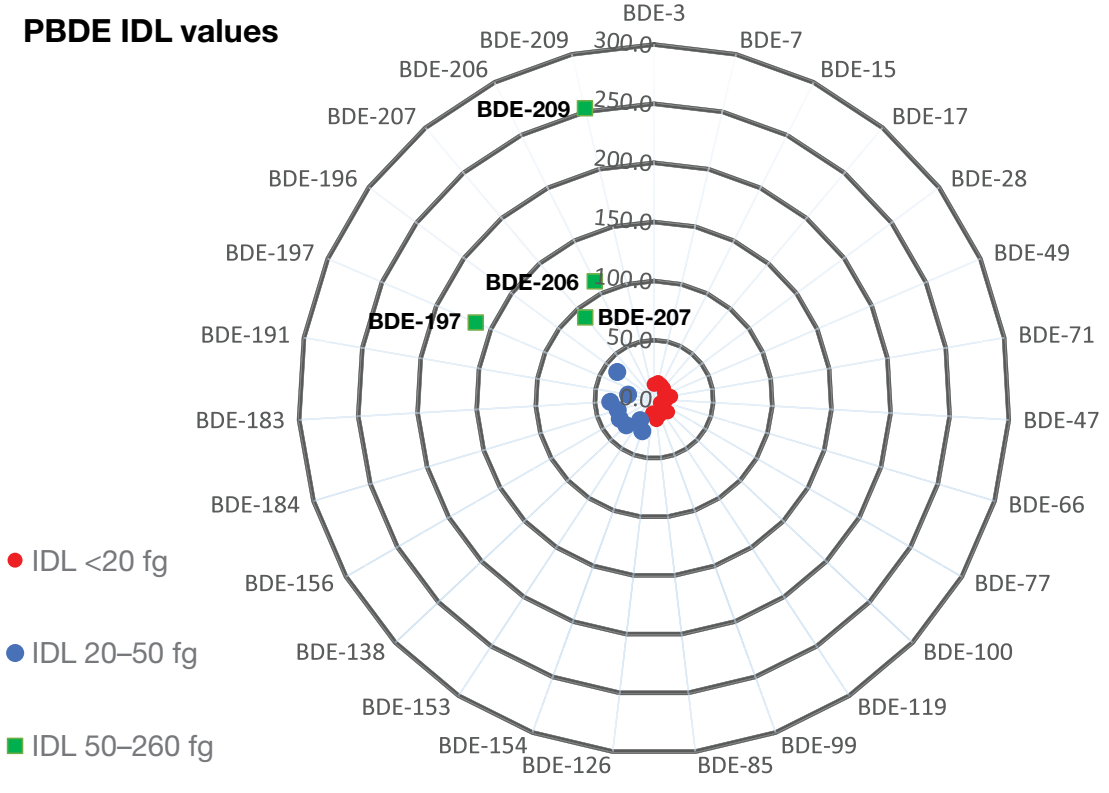


Figure 4. Calculated IDL values for 27 native PBDE congeners, statistically calculated from the results of n=14 replicate injections of the lowest serial diluted solvent standard

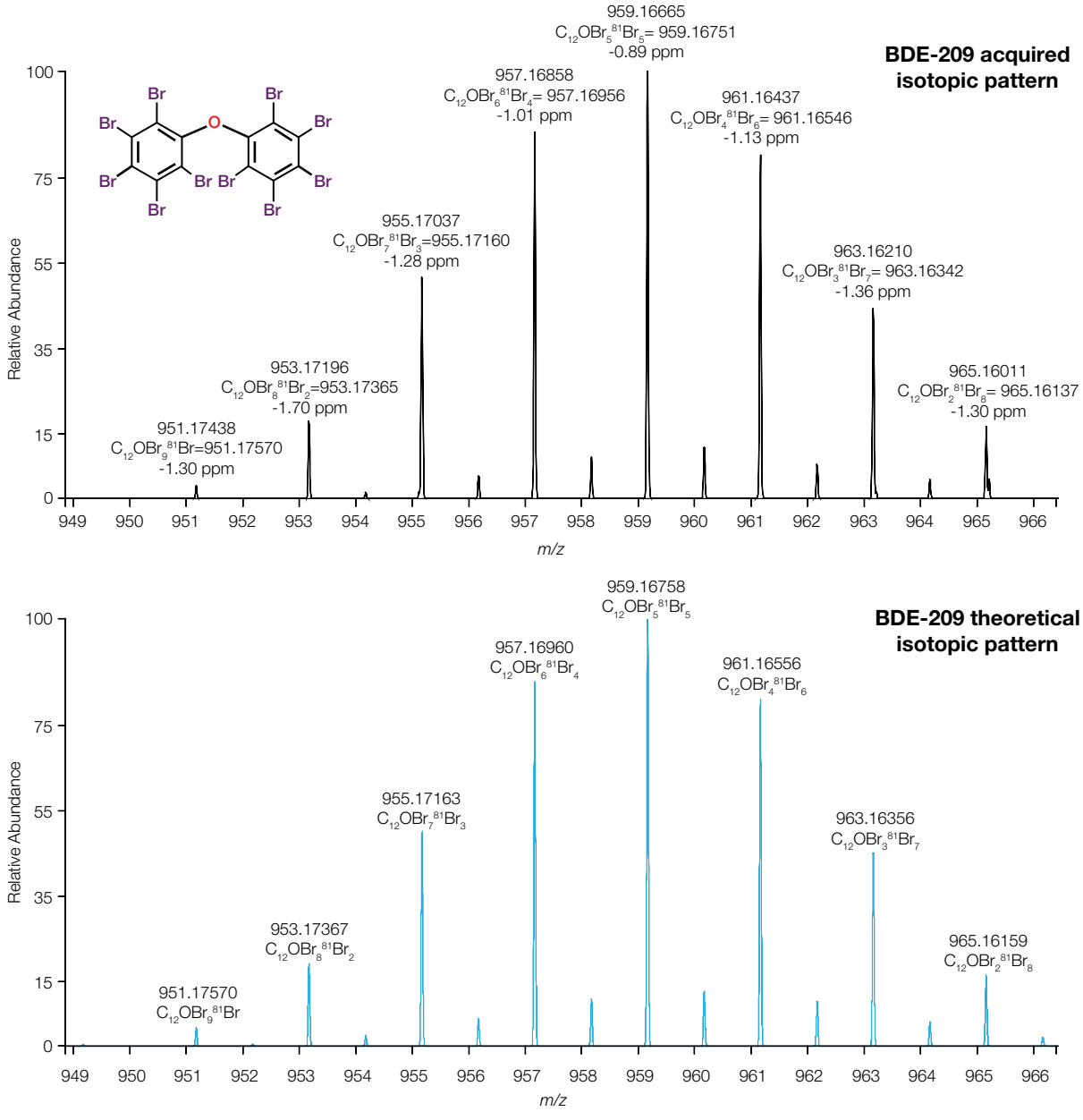
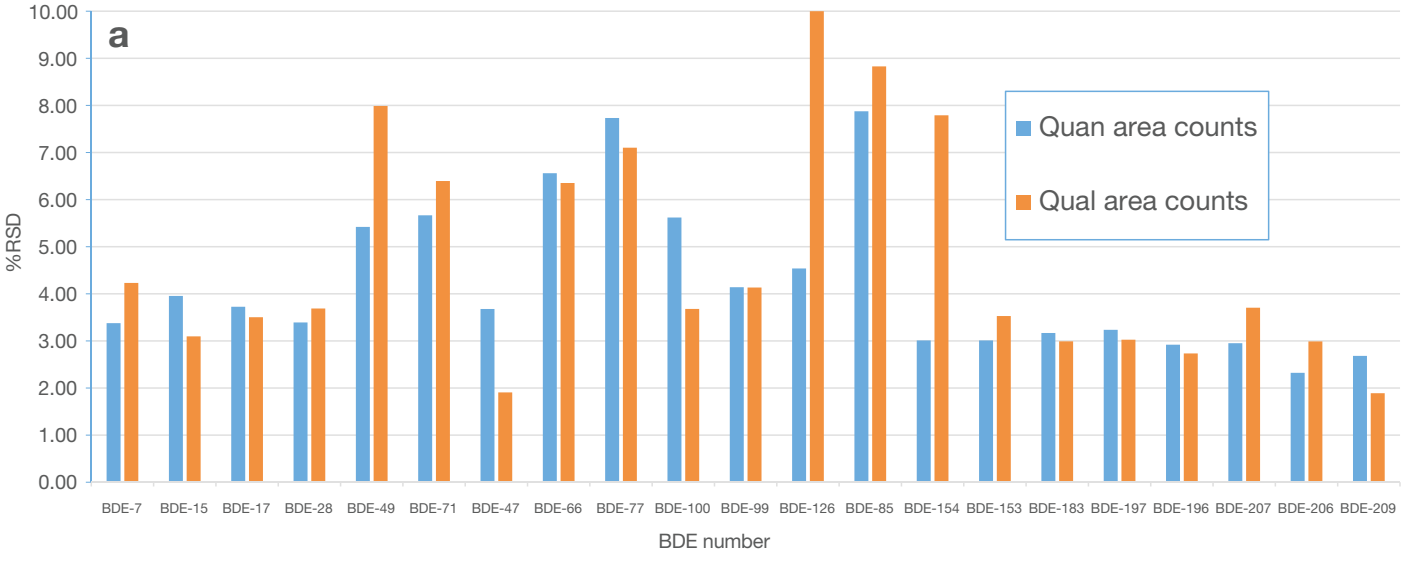


Figure 5. Comparison of mass spectra for BDE-209, acquired isotopic pattern (upper) versus the theoretical isotopic pattern (lower). Consistent <2 ppm mass accuracy obtained for each of the ion in the cluster. Annotated are the measured mass, the elemental composition, and the theoretical mass as well as the mass accuracy (ppm).

Peak area repeatability in matrix

In order to have confidence in routine PBDE quantitation results achieved, stability of responses in matrix is critical. Repeatability of PBDE responses in matrix were accessed by carrying out n=12 repeat injections of a filter dust sample extract. Excellent repeatability was obtained as shown in Figure 6a, with %RSD for quantification and qualifier peak area counts between 2% and 10% for all identified congeners, and Figure 6b, overlaid EICs (*m/z* 799.33995) for BDE-209.



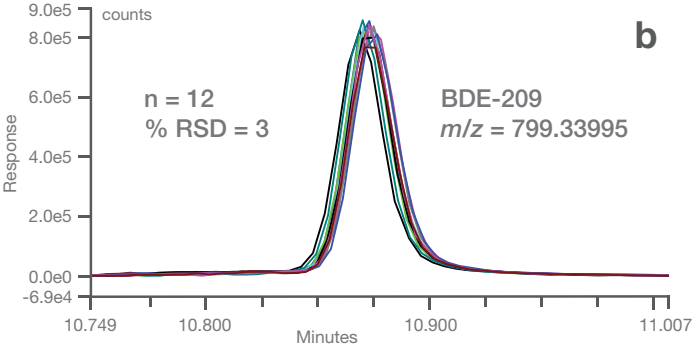


Figure 6. Replicate injections (n=12) of a filter dust sample, a) quantification and qualification area counts %RSD values for identified congeners, b) overlaid EICs (*m/z* 799.33995) for BDE-209

Linearity of response

To assess compound linearity, five calibration levels (pg on column 1 to 400 for mono- to penta-BDEs, 2 to 800 for hexa- to octa-BDEs, and 5 to 2000 for nona- to deca-BDEs) were quantified using isotopic dilution for all the congeners considered. For all PBDEs excellent linearity was obtained with R^2 values >0.995 and residual values %RSD <13% (Figure 7). Example calibration curves for BDE-209 and BDE-71 are shown in Figure 8 where both the coefficient of determination (R^2) and the residual %RSD are annotated.

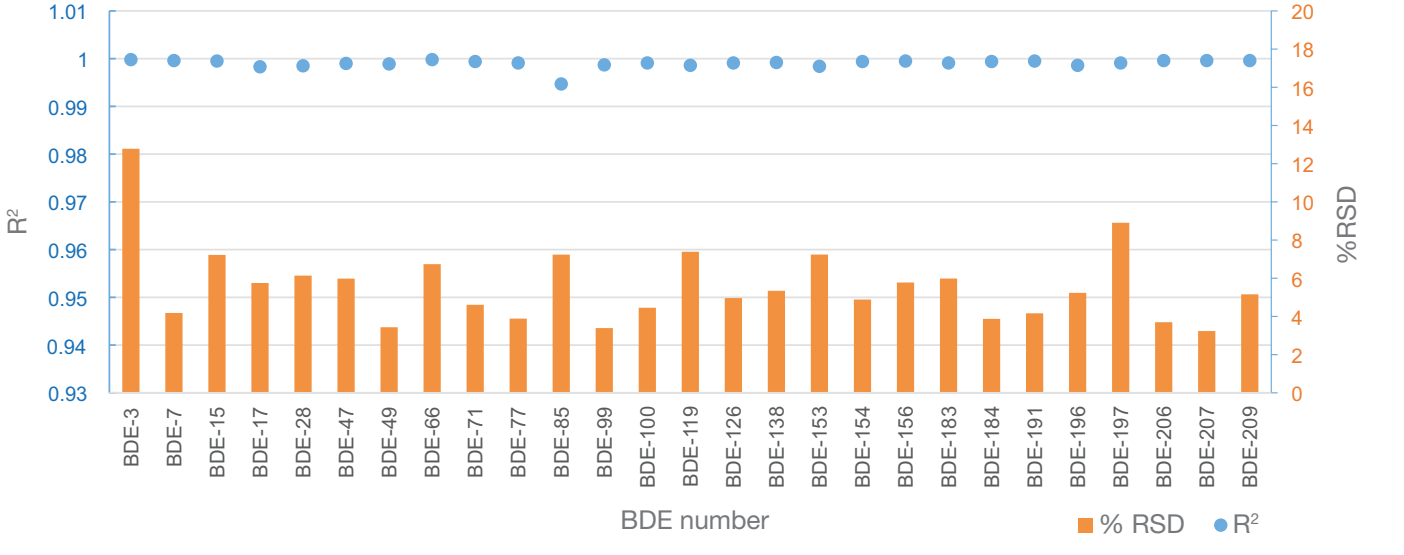


Figure 7. Coefficient of determination (left) and residuals values (%RSD) for 27 native PBDE congeners (right)

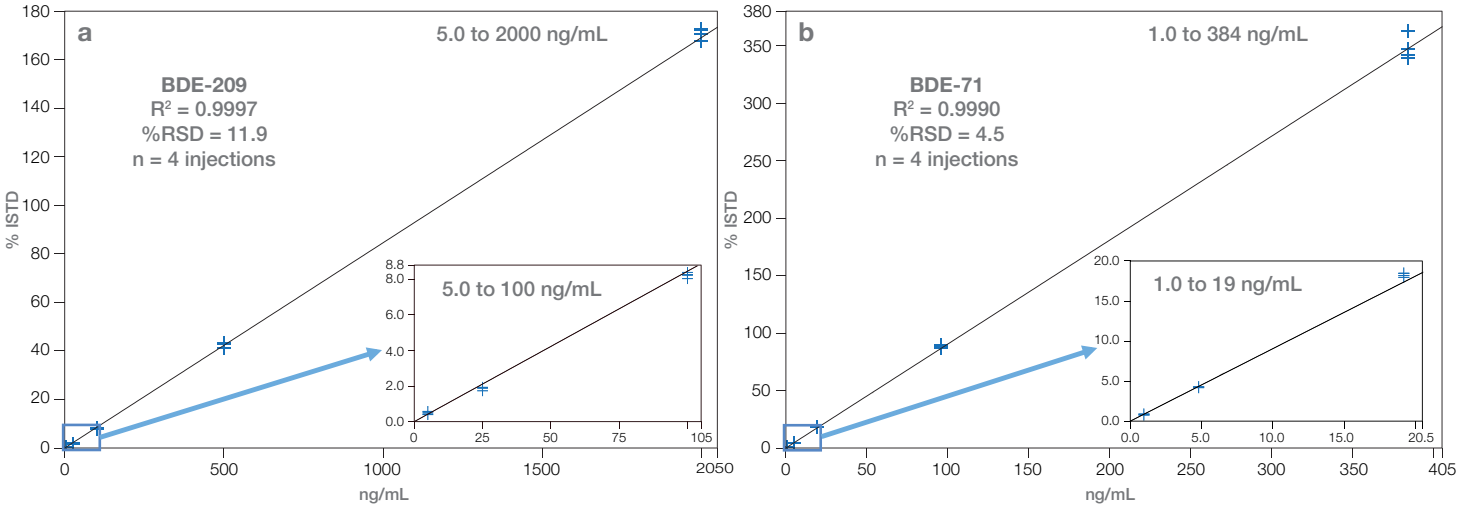


Figure 8. Example calibration curves (a) BDE-209 and (b) BDE-71, illustrating the linearity obtained. The inset calibration curves exemplify the maintained linearity for the lowest 3 calibration levels.

Sample analysis

Samples of sludge, sediment, filter dust, and air were prepared and analyzed as detailed; concentrations of the PBDEs identified are illustrated in Figure 9. The samples analyzed were extracted and quantified using isotopic dilution, using the mass-labelled PBDE surrogate standards, added to the sample prior to extraction as internal standards, and the mass-labelled PBDE recovery standard added to the extract prior to analysis as a syringe recovery standard.

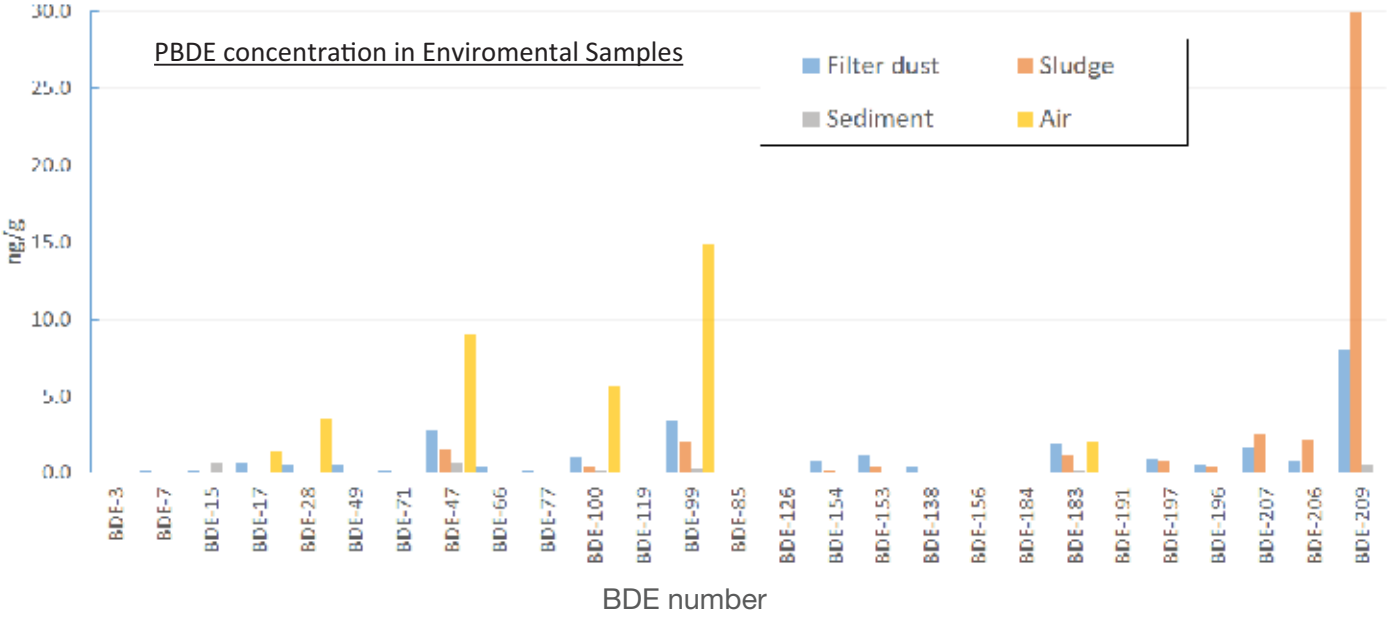


Figure 9. Calculated concentration of PBDEs, extracted and quantified from filter dust, sludge, sediment and air samples, thus illustrating the predominant PBDE congeners identified in the analyzed sludge samples as BDE-209, 206, 207, and 99, filter dust samples as BDE-209, 47, and 99, air samples as BDE-99, 47, and 100, and sediment samples as BDE-15, 47, and 99

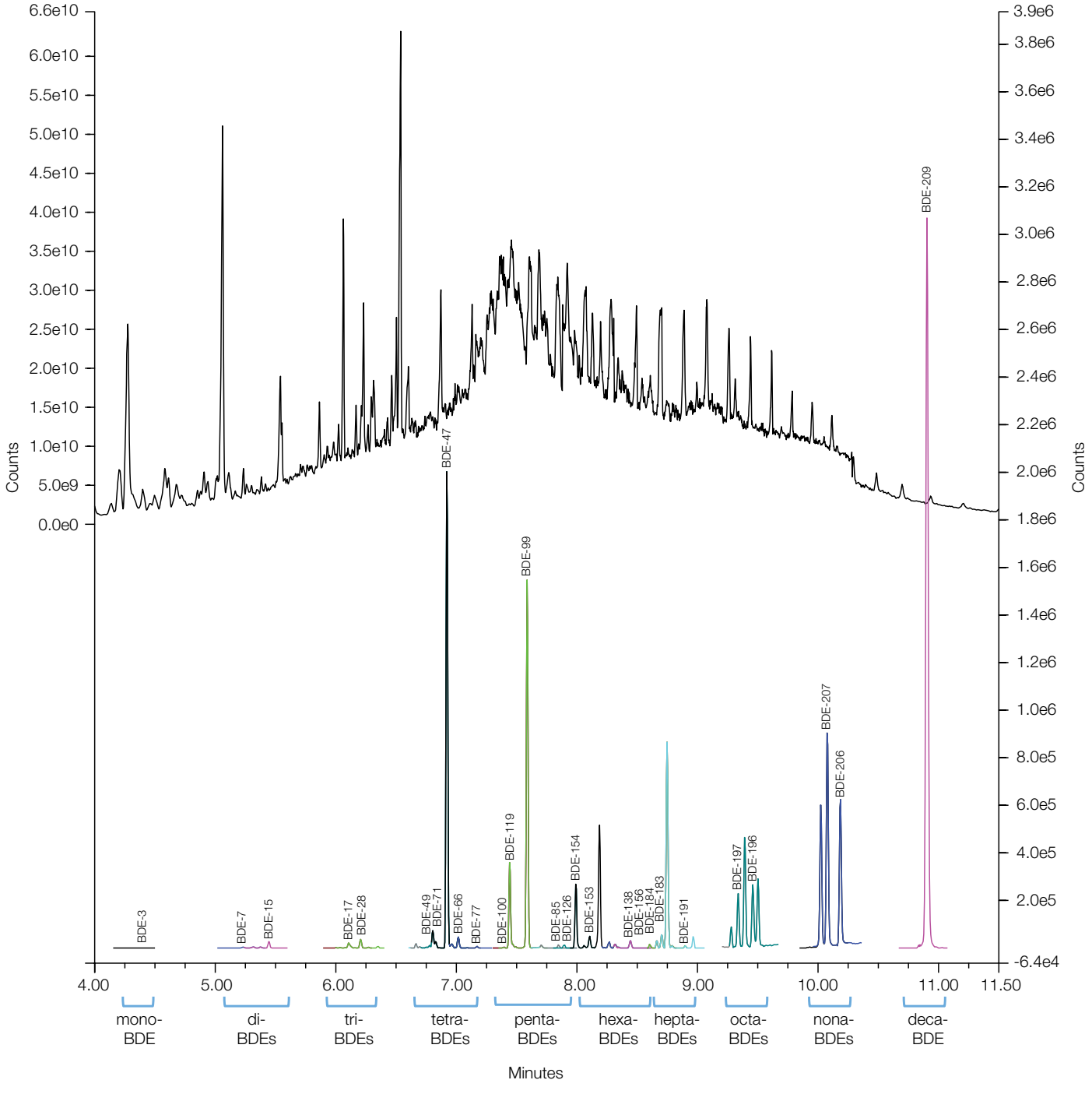


Figure 10. Sludge sample chromatograms: (upper) TIC full scan; (lower) EICs for the native PBDE congeners identified in the sample

An example of the complexity of extracted samples is shown as a total ion chromatogram (TIC) versus overlaid PBDEs EICs for a sludge sample (Figure 10), where the predominant PBDE congeners detected were BDE-209, 207, 206, 99, 47, and 183. TIC and PBDE EICs signal intensities (Y-axis) were normalized to simplify the visual comparison.

These results achieved demonstrate excellent selectivity and sensitivity for the analysis of PBDEs even in the most complex samples. Moreover, the routine high resolution of the Exactive GC offers excellent selectivity in difficult matrices, and the mass accuracy obtained allows for unambiguous identification and elemental composition confirmation of chemical contaminants.

Selectivity in matrix

The selectivity of the established method can be illustrated considering the lowest level standards, for BDE-28 and 17 (1 ng/mL, 1 pg on column), identified in a sludge sample at a similar level (Figure 11).

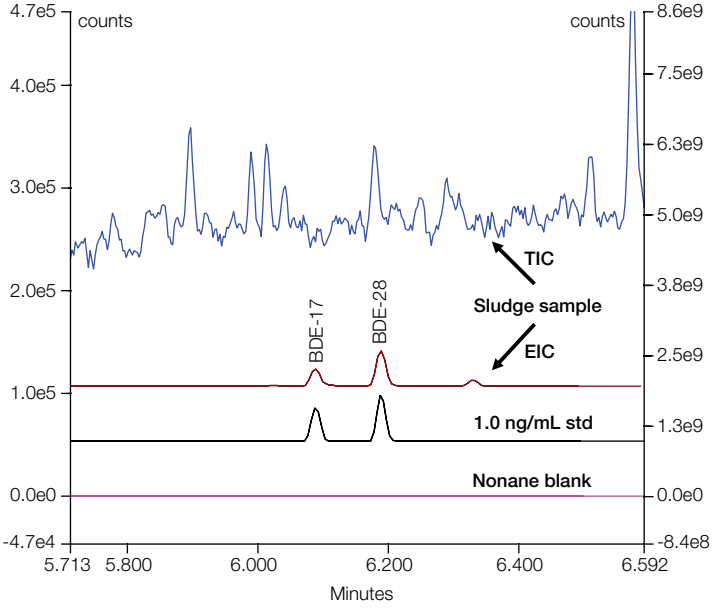


Figure 11. Overlaid EICs for BDE-17 and BDE-28 (left), in 1.0 ng/mL standard, an extracted sludge sample at a similar level, and a nonane blank. In addition, the TIC for the extracted sludge sample (right).

Conclusions

- The results of this study demonstrate that the Exactive GC Orbitrap GC-MS coupled with a TRACE 1310 GC system provides an excellent solution for routine quantification of PBDEs in complex environmental samples.
- The predominant PBDE congeners identified, confirmed, and quantified in the samples were BDE-209, 206, 207, and 99 in sludge, BDE-209, 47, and 99 in filter dust, BDE-99, 47, and 100 in air, and BDE-15, 47, and 99 in sediment.
- Using a TraceGOLD TG-PBDE 15 m capillary column, good chromatographic separation in <11 minutes for all the PBDE congeners was achieved, with excellent chromatographic resolution of the critical pair (BDE-49 and BDE-71).
- Outstanding peak area repeatability of PBDE responses in matrix with RSD% for quantification and qualifier peak area counts between 2% and 10% for all identified congeners, an important analytical parameter for routine GC-MS workflows.
- Compound linearity was demonstrated with R^2 >0.995 and residual values RSD% <13%, over five calibration levels.
- All PBDEs were detected in the lowest calibration standard, 1.0 ng/mL for mono- to penta-BDEs, 2.0 ng/mL for hexa- to octa-BDEs, and 5 ng/mL for nona- to deca-BDEs. Instrumental detection limits between 6 and 250 fg on column were achieved for the PBDEs targeted.
- Chromeleon CDS software offers an ideal solution for the targeted isotopic dilution quantification of PBDEs in environmental samples with user-friendly data processing and reporting features.

Acknowledgment

J. Parera wants to acknowledge Juan de la Cierva – Formación research fellowship (FJCI-2015-26722) from the Spanish Ministry of Economy and Competitiveness.

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Appendices

Appendix A. Details of 27 native PBDE congeners analyzed, including BDE number, chemical formula, CAS number, and calibration range

BDE number	Native BDEs	Chemical formula	CAS number	Calibration range (ng/mL)
3	4-Bromodiphenyl ether	$C_{12}H_9BrO$	101-55-3	1.0 to 400
7	2,4-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	171977-44-9	1.0 to 400
15	4,4'-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	2050-47-7	1.0 to 400
17	2,2',4-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	147217-75-2	0.96 to 384
28	2,4,4'-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	41318-75-6	1.0 to 400
47	2,2',4,4'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	5436-43-1	1.0 to 400
49	2,2',4,5'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	243982-82-3	1.0 to 400
66	2,3',4,4'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	189084-61-5	1.0 to 400
71	2,3',4',6-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	189084-62-6	1.0 to 400
77	3,3',4,4'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	93703-48-1	1.0 to 400
85	2,2',3,4,4'-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	182346-21-0	1.0 to 400
99	2,2',4,4',5-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	32534-81-9	1.0 to 400
100	2,2',4,4',6-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	189084-64-8	1.0 to 400
119	2,3',4,4',6-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	189084-66-0	1.0 to 400
126	3,3',4,4',5-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	366791-32-4	1.0 to 400
138	2,2',3,4,4',5-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	446254-95-1	2.0 to 800
153	2,2',4,4',5,5'-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	68631-49-2	2.0 to 800
154	2,2',4,4',5,6'-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	207122-15-4	2.0 to 800
156	2,3,3',4,4',5-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	405237-85-6	2.0 to 800
183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	207122-16-5	2.0 to 800
184	2,2',3,4,4',6,6'-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	117948-63-7	2.0 to 800
191	2,3,3',4,4',5',6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	446255-30-7	2.0 to 800
196	2,2',3,3',4,4',5,6'-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	446255-39-6	2.0 to 800
197	2,2',3,3',4,4',6,6'-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	117964-21-3	2.0 to 800
206	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether	$C_{12}HBr_9O$	63936-56-1	5.0 to 2000
207	2,2',3,3',4,4',5,6,6'-Nonabromodiphenyl ether	$C_{12}HBr_9O$	437701-79-6	5.0 to 2000
209	Decabromodiphenyl ether	$C_{12}Br_{10}O$	1163-19-5	5.0 to 2000

Appendix B. Details of 16 ^{13}C-labelled PBDEs internal standards, including BDE isomer number, chemical formula, CAS number, and concentration (suffix "L" indicates mass-labelled)

BDE isomer number	^{13}C labelled PBDEs	Chemical formula	Concentration (ng/mL)
3L	4-Bromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_9BrO$	100
15L	4,4'-Dibromo[$^{13}C_{12}$]diphenyl ether	$^{13}C1_2H_8Br_2O$	100
28L	2,4,4'-Tribromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_7Br_3O$	100
47L	2,2',4,4'-Tetrabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_6Br_4O$	100
79L	3,3',4,5'-Tetrabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_6Br_4O$	100
99L	2,2',4,4',5-Pentabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_5Br_5O$	100
100L	2,2',4,4',6-Pentabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_5Br_5O$	100
126L	3,3',4,4',5-Pentabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_5Br_5O$	100
138L	2,2',3,4,4',5-Hexabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_4Br_6O$	200
153L	2,2',4,4',5,5'-Hexabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_4Br_6O$	200
154L	2,2',4,4',5,6'-Hexabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_4Br_6O$	200
183L	2,2',3,4,4',5',6-Heptabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_3Br_7O$	200
197L	2,2',3,3',4,4',6,6'-Octabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_2Br_8O$	200
206L	2,2',3,3',4,4',5,5',6-Nonabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}HBr_9O$	500
207L	2,2',3,3',4,4',5,6,6'-Nonabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}HBr_9O$	500
209L	Decabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}Br_{10}O$	500

Appendix C. Details of the targeted-SIM inclusion list, listing for each entry the mass (*m/z*), start and end times, and PBDEs

Mass (*m/z*)	Start time (min)	End time (min)	BDE number
260.02339	4.00	4.50	3L, 3
327.89164	4.50	5.60	7, 15
339.93186	4.50	5.60	15L
405.80214	5.60	6.60	17, 28
417.84237	5.60	6.60	28L
485.71063	6.60	7.30	47, 49, 66, 71, 77
497.75084	6.60	7.30	47L, 79L
563.62113	7.30	8.00	85, 99, 100, 119, 126
575.66135	7.30	8.00	99L, 100L, 126L
483.69498	7.80	8.62	138, 153, 154, 156
495.73518	7.80	8.62	153L, 154L, 138L
561.60525	8.58	9.20	183. 184, 191
573.64569	8.58	9.20	183L
641.51390	9.20	9.70	196, 197
653.55416	9.20	9.70	197L
719.42446	9.70	10.40	206, 207
731.46467	9.70	10.40	207L, 206L
799.30000	10.40	12.50	209
811.30000	10.40	12.50	209L

Appendix D. PBDE retention times, quantification and confirming ions, and ion ratio averages and ranges

BDE number	RT (min)	Quantification ion	Confirming ion	Ion ratio average	Ion ratio range (±15%)	
BDE-3	4.35	249.98108	247.98313	60	51	69
BDE-7	5.21	327.89164	325.89364	50	43	58
BDE-15	5.43	327.89164	325.89364	49	42	56
BDE-17	6.09	405.80214	407.80014	74	63	85
BDE-28	6.19	405.80214	407.80014	95	81	109
BDE-47	6.92	485.71063	783.71264	68	58	78
BDE-49	6.78	485.71063	783.71264	68	58	78
BDE-66	7.00	485.71063	783.71264	68	58	78
BDE-71	6.84	485.71063	783.71264	66	56	76
BDE-77	7.14	485.71063	783.71264	67	57	77
BDE-85	7.81	563.62113	565.61912	99	84	114
BDE-99	7.54	563.62113	565.61912	100	85	115
BDE-100	7.40	563.62113	565.61912	96	81	110
BDE-119	7.45	563.62113	565.61912	98	83	112
BDE-126	7.84	563.62113	565.61912	99	84	114
BDE-138	8.40	483.69498	481.69699	66	56	75
BDE-153	8.14	483.69498	481.69699	67	57	77
BDE-154	7.95	483.69498	481.69699	67	57	77
BDE-156	8.50	483.69498	481.69699	68	58	78
BDE-183	8.71	561.60525	563.60321	102	87	118
BDE-184	8.62	563.60315	565.60120	48	41	55
BDE-191	8.85	561.60525	563.60321	100	84	116
BDE-196	9.46	641.51390	639.51595	75	64	86
BDE-197	9.35	641.51390	639.51595	73	62	84
BDE-206	10.15	719.42446	721.42000	96	82	111
BDE-207	10.04	719.42446	721.42280	99	84	113
BDE-209	10.86	799.33295	797.33497	80	68	91

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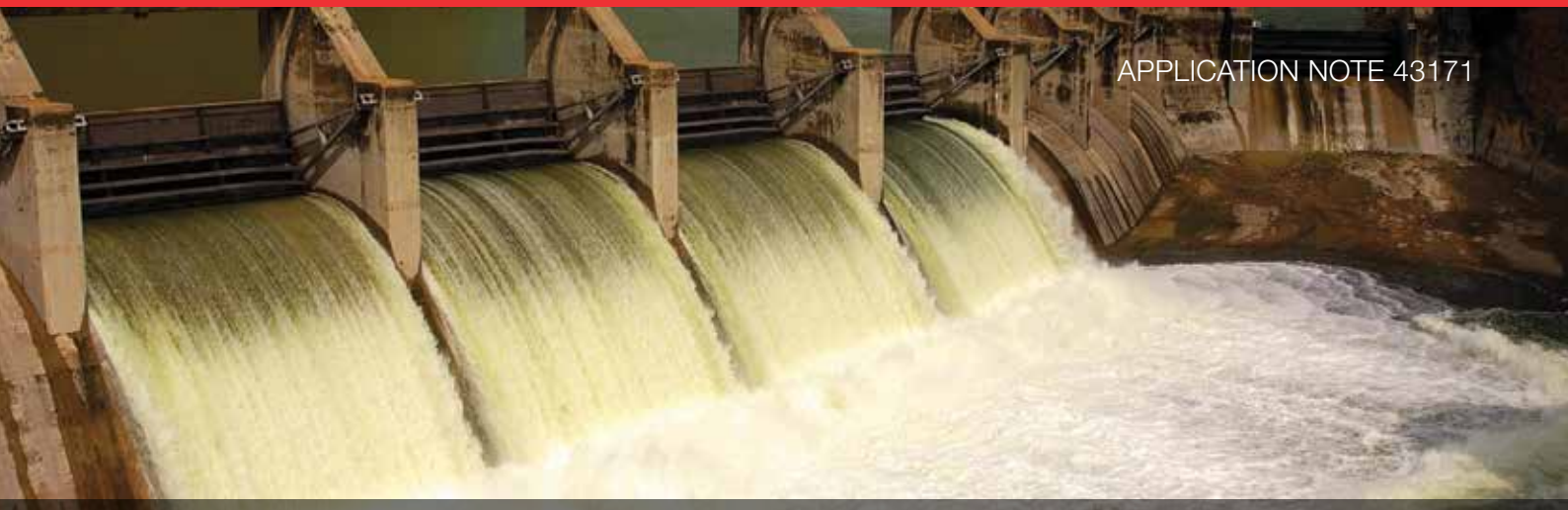

APPLICATION NOTE 43171

EU water analysis using the Thermo Scientific iCAP 7400 ICP-OES Duo

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Keywords

Environmental, EU, Waste, Water, Water Framework Directive

Goal

This application note describes the trace elemental water analysis requirements of laboratories within the European Union (EU) and how the Thermo Scientific iCAP 7400 ICP-OES Duo can be used to perform this analysis simply but to high quality standards.

Introduction

Within the EU, there are 3 types of water samples that require analysis; drinking waters, natural waters and waste waters. Each of these water types is regulated by different legislations under both European and national laws. These regulations are summarized below.

Drinking water

Drinking water analysis is performed under the guidelines of EU directive (98/83/EC) which provides maximum contaminant levels (MCL) for water to be deemed as safe for human consumption. The required MCL limits are shown in Table 1. This legislation is EU wide and requires individual member states to make provision for the required analysis. The regulation mandates for two groups of analytes; chemical parameters which are deemed toxic or hazardous to health, and indicator parameters which affect the taste, smell or quality of water.

Table 1. MCL of chemical parameters in drinking water under EU Directive 98/83/EC.

Chemical parameters	
Element	**Limit (mg·L^{-1})**
Arsenic	0.01
Antinomy	0.005
Boron	1
Cadmium	0.005
Chromium	0.05
Copper	2
Lead	0.01
Mercury	0.001
Nickel	0.02
Selenium	0.01

Table 2. MCL of indicator parameters in drinking water under EU Directive 98/83/EC.

Indicator parameters	
Element	**Limit (mg·L^{-1})**
Aluminium	0.2
Iron	0.2
Manganese	0.05
Sulfate	250
Sodium	200

Natural waters

Natural waters cover samples from any body of water including, lakes, rivers, reservoirs and coastal waters. The requirement for analysis of these bodies of water falls under the EU Water Framework Directive (WFD) (2000/60/EC), whereby individual member states are responsible for the analysis, maintenance and cleaning of these waters, as required. The WFD demands that all bodies of water within the EU be classified as either 'good' or 'high' by 2015 (some bodies of water are exempt from the 2015 deadline). If this first deadline is not met, extra time can be given to take additional measures in order to reach the objectives at the latest in 2027. The directive lists 20 specific pollutants and 33 priority substances shown to be of major concern for European Waters; 11 of the priority substances were identified as priority hazardous substances and therefore subject to cessation or phasing out of discharges, emissions and losses. 4 of the priority substances and 6 of the specific pollutants are suitable for analysis by trace elemental analysis techniques, for which the requirement for 'good' or 'high' classification status are the same. 'High' status is derived by other analytes, such as alkalinity, biological oxygen demand (BOD) and temperature.

Environmental quality standards (EQS) for waters to be classed as 'good' or 'high', including annual averages (AA) and maximum contaminant concentrations (MAC) are stated in Directive 2008/105/EC. The AA is the mean value of all samples taken over a 12 month period and the MAC is the upper allowable limit for any individual sample. The established EQS for priority substances and specific pollutants are shown in Table 3.

Table 3. EQS for priority substances listed under EU Directive 2000/60/EC.

Element	Hardness as $CaCO_3$ mg·L^{-1}	Annual Average (AA)		Maximum Allowable Concentration (MAC) (µg·L^{-1})
		All inland surface waters (µg·L^{-1})	All other surface waters (µg·L^{-1})	
Cd (PHS)	0 – 40	<0.08	0.2	<0.45
	40 – 50	0.08		0.45
	50 – 100	0.09		0.6
	100 – 200	0.15		0.9
	>200	0.25		1.5
Hg (PHS)	n/a	0.05		0.07
Ni	n/a	4	8.6	34
Pb	n/a	1.2	1.3	14

* Priority Hazardous Substance (PHS)

Table 4. EQS for specific pollutants listed under EU Directive 2000/60/EC.

Element	Hardness as $CaCO_3$ mg·L^{-1}	Annual Average (AA)	
		Rivers and fresh water lakes (µg·L^{-1})	Transitional and coastal waters (µg·L^{-1})
As	n/a	50	25
Cr III	n/a	4.7	n/a
Cr IV	n/a	3.4	0.6
Cu	0-50	1	5
	50-100	6	
	100-250	10	
	>250	28	
Fe	n/a	1000	1000
Zn	0-50	8	40
	50-100	50	
	100-250	75	
	>250	125	

Waste waters

There are currently no European wide guidelines or legislation concerning the disposal and cleaning of waste waters. The environmental agencies and departments of each member state e.g. Environment Agency (EA) in the UK, Agence de l'Environnement et de la Maîtrise de l'Energie (ADEME) in France, Umweltbundesamt (UBA) in Germany etc., are responsible for the legislation, regulation and governance of domestic, commercial and industrial waste waters. Due to the wide and varying range of legislation, the elements selected for analysis in this application note are those covered by the Environment Agency's Monitoring Certification Scheme (MCERTS) certification required in the United Kingdom.

Instrumentation

The Thermo Scientific™ iCAP™ 7400 ICP-OES Duo instrument was used for this mixed analysis of water samples. The Duo instrument was selected as the axial plasma view allows for best sensitivity and detection limits, while the radial plasma view can be used for an increased linearity. A standard aqueous sample introduction kit was used, the components can be seen in Table 6.

Sample preparation

Three water samples were sourced locally to represent each of the sample types, a drinking water, natural (river) water and waste water. These samples were passed through a 0.45 µm filter and preserved with concentrated nitric acid (TraceMetal™ grade, Fisher Chemicals, Loughborough, UK) to contain a final concentration of 2% v/v. Calibration standards were prepared using single element 1000 mg·L^{-1} stock solutions (Fisher Chemicals, Loughborough, UK), in order to match the required range of analysis. Quality control standards were prepared from independently sourced 1000 mg·L^{-1} solutions (SPEX CertiPrep®). These solutions were acid matched to the preserved samples and their final concentrations can be seen in Table 5.

Method development

Method development is an easy step when using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. A LabBook was set up using the acquisition parameters also given in Table 6.

Table 6. Instrument and acquisition parameters.

Parameter	Setting
Pump Tubing (Standard Pump)	Sample Tygon® orange/white Drain Tygon® white/white
Spray Chamber	Glass cyclonic
Nebulizer	Glass concentric
Center Tube	2.0 mm
Pump Speed	50 rpm
Nebulizer Gas Flow	0.5 L·min^{-1}
Auxiliary Gas Flow	0.5 L·min^{-1}
Coolant Gas Flow	12 L·min^{-1}
RF Power	1150 W
Exposure Time	UV 10 s, Vis 5 s

The analytical wavelengths, plasma views and internal standard wavelengths used can be seen in Table 7, along with the method detection limits (MDL) achieved. The MDLs were calculated by analyzing a blank with seven replicates and multiplying the standard deviation by 3, this was performed over 3 days and an average taken. Internal standard wavelengths were matched to analyte wavelengths by viewing mode (axial/radial view and ultraviolet/visible emissions).

Table 5. Calibration standards and quality control solution concentrations.

Solution name	STD 0	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	Initial Calibration Verification (ICV)	Continuing Calibration Verification (CCV)
Concentration (mg·L^{-1})	0	0.001	0.01	0.1	1	10	100	1	5
Elements	n/a	As, Cd, Hg, Pb, Sb, Se	Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, S, Sr, Ti				Ca, Fe, K, Mg, Na, S	Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, S, Sr, Ti, Tl, V, Zn	

Table 7. Acquisition parameters and MDL.

Element	Wavelength (nm)	Plasma view	Internal standard (nm)	MDL ($\mu g \cdot L^{-1}$)
Ag	328.068	Axial	Y 371.030	0.85
Al	396.152	Radial	Y 371.030	15
As	189.042	Axial	Y 224.306	2
B	208.959	Axial	Y 224.306	0.69
Ba	455.403	Radial	Y 371.030	0.38
Be	313.042	Axial	Y 371.030	0.038
Ca	422.673	Radial	Y 371.030	19
Cd	226.502	Axial	Y 224.306	0.18
Co	228.616	Axial	Y 224.306	0.34
Cr	267.716	Axial	Y 371.030	0.57
Cu	324.754	Axial	Y 371.030	0.69
Fe	259.940	Radial	Y 371.030	4.7
Hg	194.227	Axial	Y 224.306	0.8
K	766.490	Radial	Y 371.030	60
Mg	279.553	Radial	Y 371.030	0.16
Mn	259.373	Axial	Y 371.030	0.094
Mo	202.030	Axial	Y 224.306	0.41
Na	589.592	Radial	Y 371.030	17
Ni	221.647	Axial	Y 224.306	0.34
Pb	220.353	Axial	Y 224.306	0.7
Sb	206.833	Axial	Y 224.306	2.7
Se	196.090	Axial	Y 224.306	6.6
Sn	189.989	Axial	Y 224.306	0.81
S as SO_4	180.731	Axial	Y 224.306	9.7
Sr	407.771	Radial	Y 371.030	0.16
Ti	334.941	Axial	Y 371.030	0.39
Tl	190.856	Axial	Y 224.306	2.4
V	309.311	Axial	Y 371.030	0.27
Zn	213.856	Axial	Y 224.306	0.19

When comparing these detection limits with the regulated limits for drinking and natural water, this method is appropriate for all elements, with the exception of Cd and Hg in natural waters, under the WFD (2000/60/EC). For this analysis an alternative technique should be used, such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), as provided by the Thermo Scientific™ iCAP™ RQ ICP-MS.

Results

A batch of samples was created to analyze each of the sample types in duplicate. Each of the samples was spiked at 20% of the highest concentration standard and was analyzed beside quality control standards. This analytical sequence was analyzed seven times over a period of 4 days, as required by international standard ISO/IEC 17025:2005 (general requirements for the competence of testing and calibration laboratories). The sample list is detailed in Table 8.

Table 8. Analytical sequence of sample list.

Initial Calibration Blank (ICB)
Initial Calibration Verification (ICV)
Drinking water A
Drinking water B
Drinking water spike A
Drinking water spike B
River water A
River water B
River water spike A
River water spike B
Waste water A
Waste water B
Waste water spike A
Waste water spike B
Continuing Calibration Blank (CCB)
Continuing Calibration Verification (CCV)

The calculated mean results for the ICV and CCV quality control samples are shown in Table 9. The precision, expressed as relative standard deviation and the bias, expressed in percentage terms, are also shown. All elements displayed a precision of less than 5% and a bias within 10%, which is well within the requirements of ISO/IEC 17025:2005 and MCERTS accreditation.

Table 10 shows the mean results of each sample and spike, along with the element recoveries. The spike recoveries were within 10% for all elements and sample types.

Table 9. Mean, precision and bias of quality control samples.

Element	Mean ICV n=7 ($mg \cdot L^{-1}$)	Precision (%)	Bias (%)	Mean CCV n=7 ($mg \cdot L^{-1}$)	Precision (%)	Bias (%)
Ag	1.001	4.6	0.1	4.771	3.1	-4.6
Al	0.955	3.4	-4.5	4.675	2.4	-6.5
As	0.983	2.8	-1.7	4.722	2.8	-5.6
B	1.025	3.2	2.5	4.797	4.1	-4.1
Ba	0.966	4.2	-3.4	4.673	2.3	-6.5
Be	1.082	3.4	8.2	4.963	3.9	-0.7
Ca	0.998	2.6	-0.2	5.1	4.8	2
Cd	1.022	2	2.2	4.809	2.5	-3.8
Co	1.013	2.2	1.3	4.769	2.2	-4.6
Cr	0.985	3.2	-1.5	4.711	1.8	-5.8
Cu	0.99	2.7	-1	4.841	3.8	-3.2
Fe	1.001	1.6	0.1	4.869	3.2	-2.6
Hg	1.067	4.3	6.7	5.34	3.3	6.8
K	1.004	4.1	0.4	4.825	3.5	-3.5
Mg	1.02	3	2	5.34	3.7	6.8
Mn	1.039	2.2	3.9	4.865	1.3	-2.7
Mo	0.994	1.4	-0.6	4.74	3.7	-5.2
Na	1.031	2.9	3.1	4.93	3.9	-1.4
Ni	1.021	1.9	2.1	4.783	4.4	-4.3
Pb	1.039	1.7	3.9	4.807	0.4	-3.9
Sb	0.985	0.7	-1.5	4.756	2.6	-4.9
Se	0.999	3.3	-0.1	4.751	4	-5
Sn	1.016	0.3	1.6	4.775	1.7	-4.5
SO_4	3.005	2.3	0.2	14.55	1.1	-3
Sr	0.997	0.8	-0.3	4.832	3.5	-3.4
Ti	1.001	0.6	0.1	4.817	2.5	-3.7
Tl	1.059	1.3	5.9	4.891	2.7	-2.2
V	0.974	0.7	-2.6	4.79	1.1	-4.2
Zn	1.041	1.1	4.1	4.884	4.3	-2.3

Table 10. Mean, spike and recovery of samples.

Element	Drinking water (n=14)			River water (n=14)			Waste water (n=14)		
	Neat ($mg \cdot L^{-1}$)	Spiked ($mg \cdot L^{-1}$)	Recovery (%)	Neat ($mg \cdot L^{-1}$)	Spiked ($mg \cdot L^{-1}$)	Recovery (%)	Neat ($mg \cdot L^{-1}$)	Spiked ($mg \cdot L^{-1}$)	Recovery (%)
Ag	-0.001	1.868	93.4	0	1.866	93.3	0.001	1.872	93.5
Al	-0.005	1.907	95.6	0.007	1.888	94.1	0.43	2.422	99.6
As	0.003	2.058	102.8	0.002	2.09	104.4	0.008	2.015	100.4
B	0.015	2.079	103.2	0.053	2.044	99.6	0.034	1.92	94.3
Ba	0.068	1.903	91.8	0.049	1.936	94.4	0.06	1.868	90.4
Be	0	2.169	108.4	0	2.16	108	0	2.159	107.9
Ca	96.4	115.9	97.4	119.6	139.3	98.3	104.7	123.6	94.8
Cd	0	2.039	102	0	2.002	100.1	0	1.883	94.2
Co	0	1.956	97.8	0	1.929	96.5	0.002	1.829	91.3
Cr	-0.001	1.935	96.8	-0.001	1.949	97.5	0.001	1.839	91.9
Cu	0.448	2.275	91.3	0.005	1.95	97.3	0.054	1.861	90.3
Fe	0.012	19.68	98.4	0.01	19.78	98.9	0.307	18.98	93.4
Hg	0	1.86	93	0	1.916	95.8	0	1.941	97
K	2.15	23.77	108.1	7.092	28.55	107.3	15.99	35.36	96.9
Mg	3.981	23.39	97	6.786	25.63	94.2	5.174	24.7	97.6
Mn	0	2.043	102.2	0.002	2.061	103	0.508	2.392	94.2
Mo	0	1.999	100	0.001	1.977	98.8	0	1.919	95.9
Na	11.36	32.93	107.9	33.54	53.12	97.9	140.7	161.2	102.3
Ni	0.009	1.971	98.1	0.004	1.94	96.8	0.015	1.837	91.1
Pb	0.004	1.97	98.3	0.001	1.916	95.8	0.007	1.891	94.2
Sb	0	2.014	100.7	-0.001	1.989	99.5	-0.001	1.889	94.5
Se	-0.003	2.182	109.3	0.003	2.168	108.2	0.007	2.192	109.2
Sn	-0.001	1.999	100	-0.001	1.955	97.8	0.003	1.807	90.2
SO_4	30.01	88.62	97.7	79.03	136	95	279.3	337.1	96.2
Sr	0.312	2.328	100.8	0.548	2.485	96.8	0.235	2.378	107.2
Ti	-0.002	2.011	100.6	-0.002	2.028	101.5	0.015	1.908	94.7
Tl	0	1.975	98.7	0	1.915	95.8	-0.009	1.86	93.5
V	0.008	2.023	100.7	0.015	2.047	101.6	0.012	1.939	96.3
Zn	0.232	2.253	101.1	0.002	2.122	106	0.04	2.014	98.7

Conclusion

The data acquired from this method demonstrate the performance of the Thermo Scientific iCAP 7000 Plus Series ICP-OES instruments in analyzing water samples within the required regulations, with the exception of cadmium and mercury in natural waters under the EU Water Framework Directive (2000/60/EC). Both cadmium and mercury would typically be analyzed by ICP-MS.

The high resolution spectrometer along with the user-friendly Inter-Element Correction (IEC) function of Qtegra ISDS Software means that all interferences are either removed or compensated for automatically. This allows for simplified routine analysis and high confidence in results. The intelligent uptake and rinse function can be used to optimize uptake and washout times on a sample to sample basis, minimizing both analysis time and carryover effects.

The spike recovery data demonstrates that this method can be used to perform the analysis of all water sample types in a single sequence, without the need to optimize individual methods. The precision and bias requirements, for laboratory accreditation, can be met easily with the minimum of method development time. Qtegra ISDS Software can automatically control and perform the QC procedures required for compliance with ISO/IEC 17025:2005 and Good Laboratory Practice (GLP).

Emerging contaminants in water and wastewater

Around the world, water quality standards aim to protect water bodies from pollutants and, to a large extent, standards and regulations have done what they set out to do. We have come a long way since physician John Snow and Reverend Henry Whitehead identified a contaminated public water pump as the source of a Cholera outbreak in London in 1854. Although Snow's theories of sewage contamination and water-borne disease were not immediately accepted and it was decades before the Vibrio cholerae bacteria was identified, this discovery was a pivotal step along the road to modern sanitation and water treatment. We have been trying to identify, map, monitor, and analyze water pollutants ever since.

Fast-forward about 100 years. The Federal Water Pollution Control Act of 1948 was the first major U.S. law to address water pollution and was later amended into the Clean Water Act in 1972, which continues to govern discharges of pollutants and quality standards for surface waters of the United States. Similarly, key pieces of legislation governing water pollution throughout the United Kingdom can trace their roots back to the 1970s. But times have changed, and nearly 50 years after some of the most important pieces of federal legislation regarding water pollution were enacted, so have contaminants and our ability to detect them.

Chemical specific pollutants from point-sources are the low-hanging fruit, and that's exactly what these laws went after. In the U.S., although there is debate over the Clean Water Act's extent of success, federal water quality standards have successfully decreased the water pollutants that it targeted[1]. Most measures of water pollution have decreased, including the proportion of waters deemed unfishable. Wastewater treatment plants that received federal grants to improve treatment did in fact reduce water pollution when pollutants upstream and downstream of these plants were compared, another indicator of long-term success. Waterways, like the infamous Cuyahoga River in Cleveland, Ohio, are no longer polluted to the extent that they catch fire.

Emerging contaminants

But there's a new kid in town. Or rather, new kids. They are less conspicuous, they don't set rivers ablaze. They are emerging contaminants, and they've been left unregulated. Emerging contaminants, also called Contaminants of Emerging Concern (CEC), are chemicals or other substances that have no regulatory standard because their presence and significance has only recently been discovered and evaluated, thanks to advances in science and improved analytical detection levels[2]. So, they aren't necessarily "new", either. The European Commission's NORMAN project lists several thousands of compounds and maintains the largest database on emerging contaminants worldwide, which currently contains about ten million data records for more than 500 emerging substances[3]. In general, there are five main types of emerging contaminants:

1. Endocrine-disrupting chemicals (EDCs) – Natural and synthetic estrogen, androgens, and other chemicals

capable of modulating normal hormonal functions and steroidal synthesis in aquatic organisms.

2. Micro- and nanomaterials – Microplastics, carbon nanotubes, and other nano-scale particulates with at least one dimension that is between 1 and 100 nanometers.

3. Persistent organic pollutants (POPs) - Polybrominated diphenyl ethers (PBDEs) used in flame retardants, furniture foam, plastics, etc., and other global organic contaminants such as perfluorinated organic acids.

4. Pharmaceutical and personal care products (PPCPs) – Human prescribed drugs, over-the-counter medications, bactericides, sunscreens, cosmetics, fragrance, and other daily-use hygiene products.

5. Veterinary medicines – Antimicrobials, antibiotics, antifungals, and growth promotors and hormones.

Emerging contaminants come from a variety of sources, but much like Vibrio cholerae thriving in 19th century cesspools, they end up where we send our sewage. These days, for most of us in high-income countries at least, that's at the headworks of a wastewater treatment plant. Admittedly this is better than a cesspool, but wastewater treatment plants were not designed to target these compounds and they have the potential to sneak right through over 150 years of engineering. Globally, the percentage of wastewater treated, municipal and industrial , drops with income, and about 80% of wastewater generated on Earth is discharged without any treatment at all[4]. Throw in urban and agricultural runoff and we've essentially fast-tracked contaminants to surface water and sources of drinking water. They don't even need to be sneaky about it. Have we been outsmarted?

Per- and polyfluoroalkyl substances (PFASs)

PFASs are POPs in the world of emerging contaminants. Dana Gonzalez, a Treatment Process Engineer at Hampton Roads Sanitation District (HRSD) in the United States, explains "They don't exist in nature, but we've created about 4,000 different PFAS compounds out there. We like them because they have a predominance of carbon-fluorine bonds, the strongest [single] carbon bond in nature, and they are great at keeping water from penetrating just about anything. They are known for their use in Aqueous Film Forming Foam (AFFF), a type of firefighting foam used widely at airports and military facilities because of its ability to smother a jet-fuel fire in minutes." It was so impressive, we started putting PFAS in everything from carpets to textiles to food wrappers. Do you want it stain-repellent, water-repellent, or nonstick? Add some PFAS to it.

Other impressive qualities of PFAS include its ability to persist in the aquatic environment and its potential to bioaccumulate. Releases into the environment occur through industrial manufacturing and through use and disposal of PFAS-containing products, so there are a variety of ways people can be exposed. Exposure in humans has been associated with increased cholesterol levels, effects on the immune system and thyroid hormone function, and cancer[5]. Gonzalez' work focuses on compounds like PFASs and because she knows what she is looking for, she uses liquid chromatography - mass spectrometry (LC-MS) to get the information she needs. Mass spectrometers provide selective monitoring of known compounds, but what if you don't know what you are looking for? That is often the case with emerging contaminants, and it presents the age-old question, how do you fight an enemy you cannot see?

High resolution mass spectrometry (HRMS) is part of the answer. Liquid chromatography - high resolution mass spectrometry (LC-HRMS) and gas chromatography - high resolution mass spectrometry (GC-HRMS) are two methods that allow for the identification of compounds you don't expect. When coupled with chromatography separation techniques, HRMS measures the exact mass of each compound, detecting analytes to the nearest 0.001 atomic mass units. In comparison, normal mass spectrometry measures in integers and so is not as selective.

Techniques for contaminant analysis

As analytical technologies improve, it's all about getting to zero. But is the search for zero achievable or necessary? Some believe there are cases where it's a moot point. "We are worried about PFAS in our drinking water, but we are also breathing it in through dust and other sources," explains Chris Burbage, an Environmental Scientist at HRSD, "Or if we focus on organic pollutants, think about what can leach from our landfills and what we put on our roads. Highway runoff carries fossil fuels, road salt, and now studies are showing high concentrations of microplastics in highway runoff." We live in a chemical world, and the sources and points of contact we have with emerging contaminants are seemingly infinite. Of course, that's not to say we should throw our hands up, but in some cases exposure through one source, like drinking

water, may be negligible in the big picture. There are more than 100 million chemicals registered in the Chemical Abstracts Service (CAS), with about 4,000 new ones added every day[3]. We've got to think inclusively when it comes to managing our water resources.

Jamie Heisig-Mitchell, who leads the division overseeing environmental monitoring programs and permit reporting at HRSD, believes we need to recognize our roles. "People have lost sight that the wastewater treatment plants are not the generators [of the contaminants]. We are dealing with what society is providing. Society needs to be more thoughtful about the products we allow in our home, what we put down the drain, and what we generate for solid waste landfills. Everything we use for the sake of convenience has a cost. How do we want to manage that cost?"

Right now we are paying for it down the line, at places like wastewater treatment plants, the last line of defense between pollution and the environment. In most places, there is no last line of defense. Some emerging contaminants have been around for millennia and others are just coming online through advances in medicine and technology and consumer priorities on convenience. Snow is quoted as saying, "All that would be required to prevent the disease [cholera] would be such a close attention to cleanliness in cooking and eating, and to drainage and water supply, as is desirable at all times." Two centuries later, we know it's also the chemicals that help grow our food and make our cookware that pose a potential risk to human health. We need to stay one step ahead with research and technology that matches the products being produced. If we don't, we could face costly consequences in terms of environmental impact and human health.

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APPLICATION NOTE

Quantitative comparison of hormones in drinking water between MS/MS and Orbitrap technology

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Key Words

Contaminants of emerging concern, CEC, endocrine disrupting compound, EDC, micropollutants, EPA Method 539, Q Exactive

Goal

To demonstrate a liquid chromatography – high-resolution, accurate mass (LC-HRAM) methodology using Orbitrap™ technology as a sensitive, accurate, and reliable alternative to the use of triple quadrupoles mass spectrometers in the quantification of hormones in drinking water according to EPA guidelines.

Introduction

Increasingly, contaminants of emerging concern (CEC) including pharmaceuticals and personal care products, such as the contraceptive pill and antibiotics, are being detected at low levels in surface water. Many of these CEC are endocrine disrupting compounds (EDCs), which can alter the normal functions of hormones and cause a variety of health effects.[1,2] As a result, the United States Environmental Protection Agency (EPA) has developed EPA Method 539[3] for the Unregulated Contaminant Monitoring Rule 3 (UCMR 3) program, which collects data for contaminants suspected to be present in drinking water but that do not have health-based standards set under the Safe Drinking Water Act (SDWA).[4]

The identification and quantification of micropollutants at low concentrations requires both sensitivity and selectivity against complex matrices. Selected reaction monitoring (SRM) of precursor-product ion transitions, which makes use of a triple quadrupole mass analyzer, has been the method of choice.[5] However, other screening strategies employing full scan mode and other advanced MS/MS scan modes can potentially offer a valuable alternative to SRM based methodology due to the development of more rugged, sensitive, and selective instrumentation.

The quantitative performance of the latest generation of high-resolution instruments is comparable to that of a triple quadrupole MS, even though different scanning modes are used. Higher-resolution instrumentation also allows flexibility concerning compound identification because the experiment can be set up for targeted quantitation, screening, or both. In an Orbitrap-based instrument, the parallel reaction monitoring (PRM) mode performs most closely to a triple quadrupole mass analyzer using SRM mode. This study compares the quantitation performance between a triple quadrupole (MS/MS) to that of an Orbitrap-based detector using EPA Method 539: *Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization and Tandem Mass Spectrometry (LC-ESI-MS/MS).* All other aspects of the method including sample preservation, storage, preparation, and chromatographic separation were kept the same. The only differenc

Experimental

Sample preparation

The sample preparation is based on EPA Method 539. Any modifications and text are highlighted for clarity and discussion purposes. Five hundred milliliters of a dechlorinated sample with Omadine™ biocide was extracted through solid phase extraction (SPE) using an octadecyl (C-18) stationary phase after adding surrogates. The eluent from SPE was concentrated to dryness and then diluted to 1 mL with 50:50 methanol/water. An aliquot was injected into the LC-MS/MS after adding internal standards and quantified against the internal standard (IS).

LC-MS conditions

Under the EPA Method, flexibility is allowed for columns, eluents, and MS conditions in general. Table 1 shows the conditions optimized and used in the analysis.

Table 1. LC-MS conditions optimized and used for the experiments described.

Mass Analyzer	Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer
Mass Resolving Power	70,000 (FWHM) at *m/z* 200
Scan Mode	PRM
AGC	2e5
IT	200 ms
Isolation Window	1.0 *(m/z)*
HPLC	Thermo Scientific™ UltiMate™ 3000 RS UHPLC, binary pump, autosampler, and column heater with 100 µL sample loop
Column	Thermo Scientific™ Acclaim™ PolarAdvantage II (2.1 x 150 mm, 3 µm, 120 Å, P/N 063187)
Eluents	A) 1 mM ammonium fluoride in water B) 50:50 (v/v) acetonitrile/methanol Gradient flow at 0.3 mL/min with a 21.4 min run
Injection Volume	50 µL

EPA Method 539 uses a triple quadruple method using an SRM scan mode (also known as MRM). According to EPA Method 539, section 3.16, "MRM... a mass spectrometric technique in which a precursor ion is first isolated, then subsequently fragmented into a product ion(s). Quantitation is accomplished by monitoring a specific production." In this study, a similar set of conditions was used.

In PRM mode, a list of targeted precursor ions, retention times, and collision energies can be included in the method. When detecting a targeted ion, the system isolates that precursor ion in the quadrupole and triggers MS/MS experiments, generating MS/MS spectra that can be used for both quantitation and identification. Both the quantitation and identification are performed taking into account product ions generated after the isolation of a specific precursor ion. This operating mode is similar to an SRM (or MRM) experiment using a triple quadrupole instrument. In PRM mode, the third quadrupole is substituted with an HRAM (high-resolution, accurate mass) mass analyzer, enabling the parallel detection of all target product ions (Figure 1).

The number of scans across the chromatographic peak is dependent on the cycle time of the instrument and, therefore, on the set of conditions used (e.g., resolving power). These conditions can and should be optimized depending on the objectives of the experiment. In this case, accurate quantitation as well as unambiguous identification has been targeted. Optimized conditions can be found in Table 1.

Requirements

The EPA has strict requirements that should be met before the analysis of any sample, referred to as the Initial Demonstration of Capability (IDC). These requirements include the demonstration of low background noise, precision by analyzing four to seven extracted laboratory fortified reagent water blanks (LFB) at mid-level, the demonstration of accuracy and, finally, the demonstration of capability necessary to meet the minimum reporting limit (MRL). The percent relative standard deviation (%RSD) of the results of the replicate analyses must be ≤ 20%. The average percent recovery for each analyte must be within ± 30% of the true value.

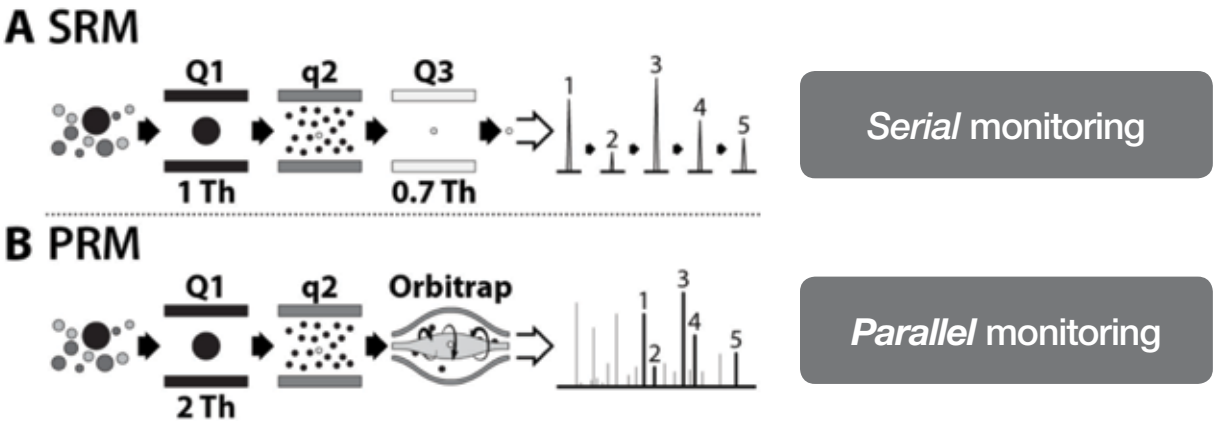

Figure 1. Schematic representation of selective reaction monitoring (SRM) mode and parallel reaction monitoring (PRM) mode.

Results and discussion

Excellent linearity has been demonstrated from a range starting at one-fourth of the MRL (Figure 2). Table 2 compares the MRL and LCMRL obtained when using both SRM and PRM modes. Tables 3, 4, and 5 summarize precision and accuracy of the method after the LC-HRAM analysis of different types of samples—reagent water spiked at different levels and UCMR3 water samples.

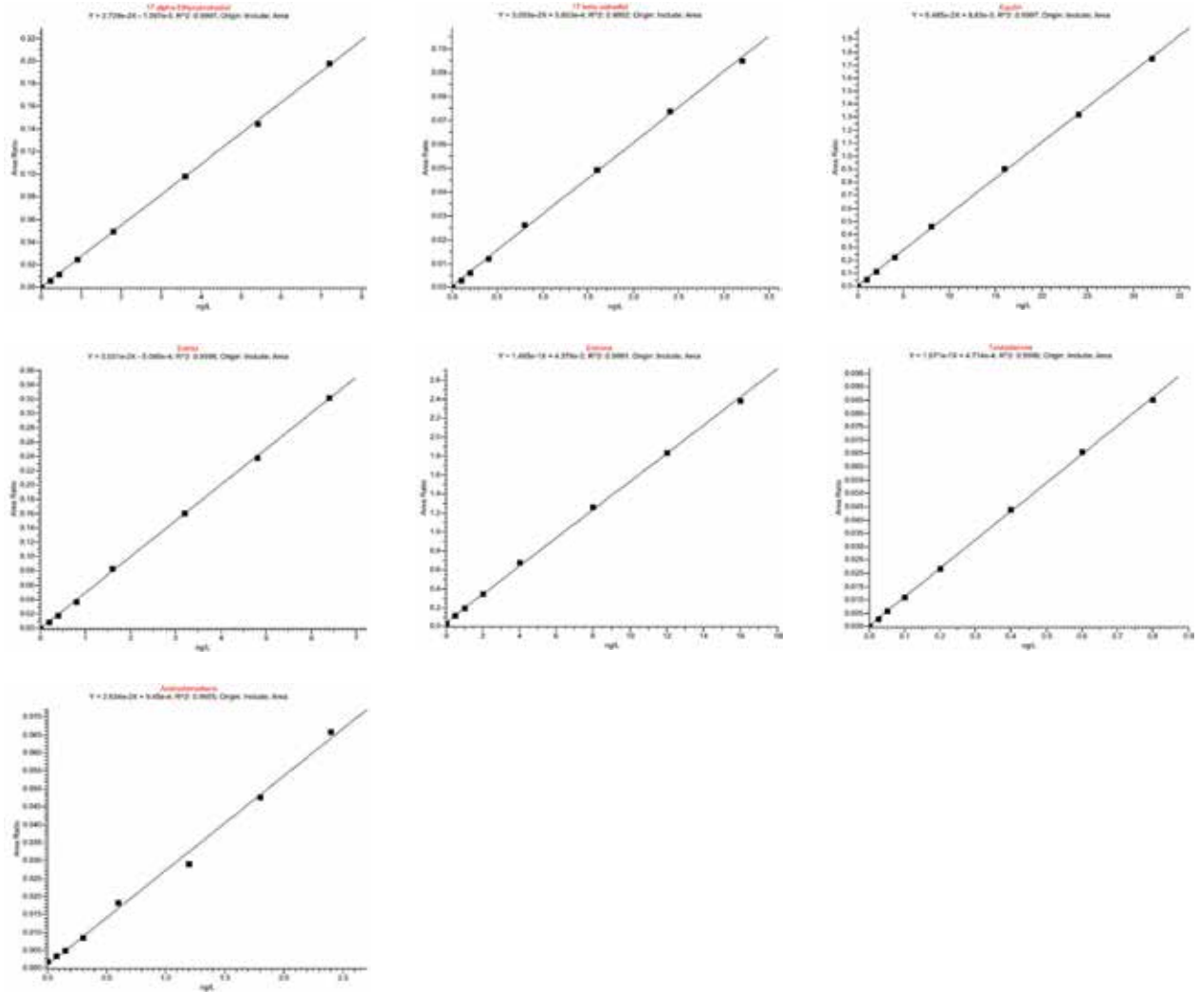

Figure 2. Calibration curves for all EPA Method 539 analytes.

Table 2. MRL and LCMRL comparison when using triple quadrupole and Orbitrap mass analyzers in reagent water preserved according to EPA Method 539.

Analyte	UCMR3 MRL (ng/L)	EPA 539 published LCMRL (ng/L)	LC-HRAM[a] LCMRL (ng/L)	LC-HRAM[a] LCMRL Calc -DL (ng/L)
17α-ethynylestradiol	0.9	1.3	Critical level 0.05[b]	0.1
17β-estradiol	0.4	0.32	0.17	0.047
equilin	4	0.28	Critical level 0.23[b]	0.48
estriol	0.8	3	0.27	0.2
estrone	2	4	0.84	0.48
testosterone	0.1	0.062	0.033	0.027
4-androstene-3,17-dione	0.3	0.37	0.19	0.08

[a]The detection limits reported in EPA Methos 539 reflect the MS/MS, Ion Trap, and Hybrid MS technology used at the time of method validation. They are shown here for reference purposes. Detection limits for newer MS/MS instruments can either be lower or higher depending on many variables including operator performance, instrumentation, sample preparation, and other factors. Thus, the lower DL for Orbitrap technology shown here demonstrate that quantitatively the results are comparable with the reported method.

[b]The critical level calculation can't find the MRL as the lowest standard wasn't low enough for exact determination. Thus a lower level spiking concentration is required to determine the LCMRL for these compounds.

As shown in Table 2, the LCMRL and DL were much lower when using LC-HRAM than the detection limits reported in EPA Method 539. This demonstrates the greater sensitivity using Orbitrap HRAM compared to the MS/MS and hybrid instruments used during method validation. In order to demonstrate method robustness, the EPA requires the demonstration of performance using a fortified matrix in blanks, reagent water, and real samples. Results are summarized in Tables 3, 4, and 5.

Table 3. LC-HRAM method: Precision and accuracy in fortified reagent water spiked at 10 x MRL.

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17α-ethynylestradiol	7.2	82%	4
17β-estradiol	3.2	84%	3
equilin	32.0	81%	3
estriol	6.4	100%	4
estrone	16.0	83%	4
testosterone	0.8	87%	5
4-androstene-3,17-dione	2.4	85%	8

n=4

Table 4. LC-HRAM method: Precision and accuracy in fortified matrix (UCMR3 water sample 1) spiked at MRL.

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17α-ethynylestradiol	0.72	95%	2
17β-estradiol	0.32	87%	1
equilin	3.20	92%	8
estriol	0.64	101%	4
estrone	1.60	95%	3
testosterone	0.08	99%	0.1
4-androstene-3,17-dione	0.24	118%	0.1

n=4

Table 5. LC-HRAM method: Precision and accuracy in fortified matrix (UCMR3 water sample 2) spiked at 10 × MRL.

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17α-ethynylestradiol	7.2	98%	3
17β-estradiol	3.2	113%	0.8
equilin	32.0	102%	0.7
estriol	6.4	103%	2.4
estrone	16.0	110%	1.7
testosterone	0.8	103%	0.3
4-androstene-3,17-dione	2.4	104%	1.4

n=4

Conclusion

The LC-HRAM methodology proved to be sensitive, accurate, reproducible, and a reliable alternative to the use of triple quadrupoles in the quantification of hormones in drinking water according to the EPA guidelines. By the use of different scanning modes within the Q Exactive MS, quantitation on precursor ions and identification of fragments ions are possible. These scanning modes are consistent with the requirements in many regulated methods and can possibly be used for compliance monitoring in place of a triple quadrupole MS. The latest LC-HRAM technology assures sensitivity and selectivity in the quantitation of known contaminants in drinking water, while potentially enabling the combination of targeted and non-targeted analysis in the same run, which cannot be accomplished using MS/MS alone.

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APPLICATION NOTE 10615

Unparalleled performance of Advanced Electron Ionization GC-MS/MS technology for the determination of nitrosamines in drinking water

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Keywords

Environmental analysis, nitrosamines, NDMA, trace analysis, gas chromatography, TSQ 9000, triple quadrupole mass spectrometry, selected reaction monitoring, drinking water, advanced electron ionization

Goal

The aim of the study was to assess the quantitative performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system with advanced electron ionization source for the analysis of nitrosamines in drinking water at low concentrations.

Introduction

Nitrosamines are semi-volatile compounds that are an emerging class of drinking water contaminants. *N*-nitrosodimethylamine (NDMA) is the main nitrosamine of concern and is classified as a potent carcinogen by the U.S. Environmental Protection Agency (EPA) due to its tumor-inducing properties through ingestion or inhalation.[1] Nitrosamines are used in various industries to manufacture cosmetics, pesticides, or rubber products. In water, nitrosamines are formed as by-products during industrial processes such as chloramination of wastewater and drinking water.[2] Due to their potency as carcinogens, nitrosamines are considered as priority pollutants, and various countries around the world have already introduced maximum acceptable concentrations of 9 ng/L and notification levels at 10 ng/L.[3,4]

GC-MS is the analytical technique of choice for nitrosamine determination and, in particular the use of triple quadrupole GC-MS/MS instrumentation has recently become popular for this application due to its high selectivity and sensitivity provided through selective reaction monitoring (SRM). High selectivity and sensitivity are required to (i) reduce interferences from matrix and background chemical ions that can result in false positive detection and erroneous quantification of nitrosamines and (ii) detect ultra-trace levels of these toxic compounds.

In this work, the analytical performance of the TSQ 9000 triple quadrupole GC-MS/MS system using the Thermo Scientific™ Advanced Electron Ionization (AEI) source was tested for the ultra-trace analysis of nitrosamines in drinking water from 17 drinking water testing facilities across Europe.

Experimental

Preparation of solvent calibration curve

To test the limit of detection (LOD) and to assess the linearity of the method, individual nitrosamine standards including NDMA d-6 surrogate (LGC Ltd, UK) were used to prepare nine calibration levels: 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10, 20, 50, and 100 pg/µL (corresponding to 0.05–100 ng/L in drinking water after concentrating ×1000 with SPE). NDPA-d14 was also spiked in as an internal standard at 25 pg/µL (corresponding to 25 ng/L in drinking water).

Preparation of samples

Solid phase extraction (SPE) was performed using activated charcoal SPE based on modified EPA 521 methodology. The summary of the SPE method can be seen in Figure 1. In addition, the limit of quantitation (LOQ) was assessed by fortifying ultra-pure water with nitrosamines at 0.1 and 0.5 ng/L (step 2). Similarly, recovery was assessed by fortifying water at 50 ng/L (step 2).

GC-MS/MS analysis

A TSQ 9000 triple quadrupole GC-MS/MS instrument equipped with an AEI source and coupled with a Thermo Scientific™ TRACE™ 1310 GC system was used. The AEI source provides a highly efficient electron ionization of analytes and a more tightly focused ion beam that leads to an unparalleled level of sensitivity.

Liquid injections of the sample extracts were performed using a Thermo Scientific™ TriPlus™ RSH autosampler and chromatographic separation was achieved by a Thermo Scientific™ TraceGOLD™ TG-1701 MS 30 m × 0.25 mm I.D. × 0.50 µm film capillary column. Additional details of instrument parameters are displayed in Table 1.

Data processing

Data were acquired using timed-SRM, processed, and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, which allows instrument control, method development, quantitative/qualitative analysis, and customizable reporting all within one platform.[5] Data review is highly customizable, allowing the user to display the information required on screen in real time and the software is FDA 21 CFR part 11 compliant.

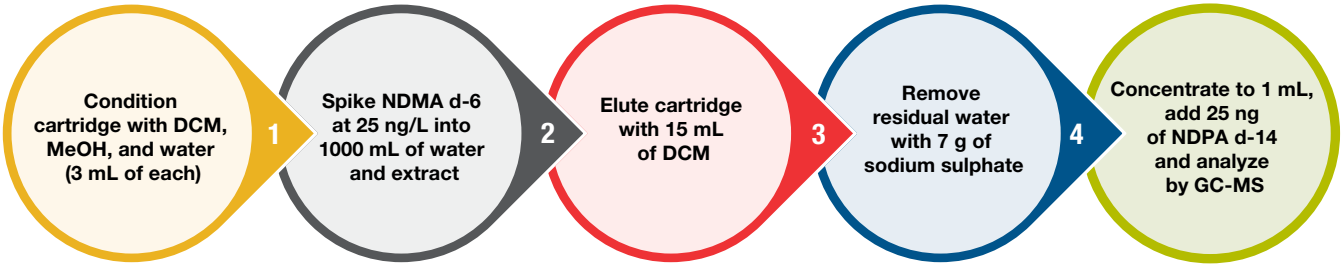

Figure 1. SPE steps used for drinking water samples

Table 1. Instrument parameters used in the drinking water analysis. A full list of consumables and instrument conditions including SRM transitions can be found in the AppsLab library.

TRACE 1310 GC system parameters					
Injection Volume:	2.0 µL				
Liner:	Restek® CarboFrit® liner (P/N 20294)				
Inlet:	240 °C				
Carrier Gas:	He, 1.3 mL/min				
Injector Injection Mode:	Splitless with surge (surge pressure 25 psi for 1.01 min, split flow 80 mL/min after 1 min)				
Column:	TraceGOLD TG-1701MS (30 m × 0.25 mm, 0.5 µm P/N 26090-2230)				
Oven Temperature Program:					
	Ramp	*RT (min)*	*Rate (°C/min)*	*Target Temperature (°C)*	*Hold Time (min)*
	Initial	0.0	-	35	1.0
	1	4.8	25.0	130	0.0
	Final	12.8	20.0	250	2.0
	Run time	12.8	-	-	-
TSQ 9000 Mass Spectrometer parameters					
Transfer Line:	250 °C				
Source Used:	Thermo Scientific™ Advanced Electron Ionization (AEI)				
Ionization Type, eV, Emission Current:	Electron Ionization (EI), 50, 100 µA				
Ion Source:	300 °C				
Acquisition Mode:	Timed SRM				
Tune Type:	AEI SmartTune				
Collision Gas and Pressure:	Argon at 70 psi				
Peak Width:	0.7 Da at FWHM (both Q1 and Q3)				

See SRM transitions in Appendix.

Results and discussion

The objective of the analysis was to test the TSQ 9000 triple quadrupole GC-MS/MS system performance for the targeted analysis of nitrosamines in drinking water samples. To accomplish this, solvent standards and real drinking water samples were analyzed.

Nitrosamines chromatography, selectivity, sensitivity, linearity, and peak area repeatability were evaluated using solvent-based standards. This was followed by validation of the method using fortification of water samples prior to SPE and concentration to assess compound LOQs and recoveries. The method was then applied to quantify nitrosamines in several drinking water samples obtained from water treatment stations across Europe.

Carryover can be a problem for this application, to assess the performance of this effect a dichloromethane (DCM) blank was injected immediately after the highest concentration standard. In Figure 2 an example extracted ion chromatogram of the highest concentration injected standard for NDMA 200 pg on column (oc) (left chromatogram) and the consecutive DCM blank (right chromatogram) demonstrates that there is no carryover.

Chromatography

All compounds were separated in less than 9 minutes, which is 3× faster than what is suggested in certain methodology such as EPA Method 521. This will allow for high sample throughput and reduced cost per analysis. Using the TraceGOLD TG-1701 MS column, good chromatographic peak shape was obtained for all compounds, even for NDMA which is particularly challenging for this analysis due to its polarity (Figure 3).

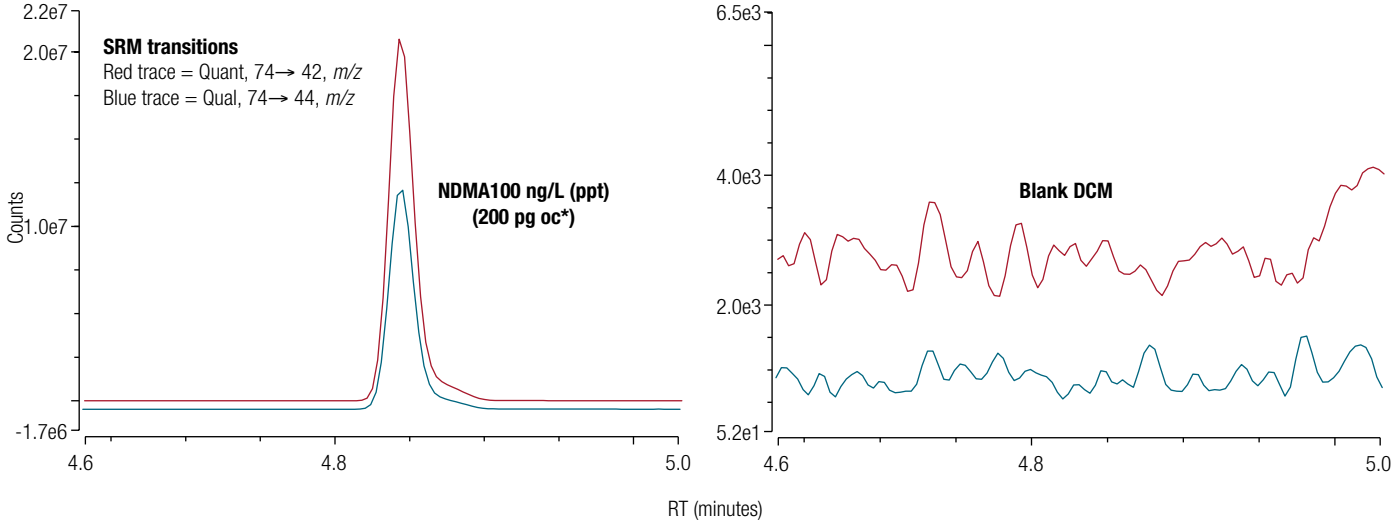

Figure 2. NDMA overlaid quantification ion and qualification ions for the highest standard in dichloromethane 100 pg/µL corresponding to 200 pg on-column (oc*) (left chromatogram) and a consecutive DCM blank (right chromatogram). Data is unsmoothed and was acquired in timed-SRM mode.

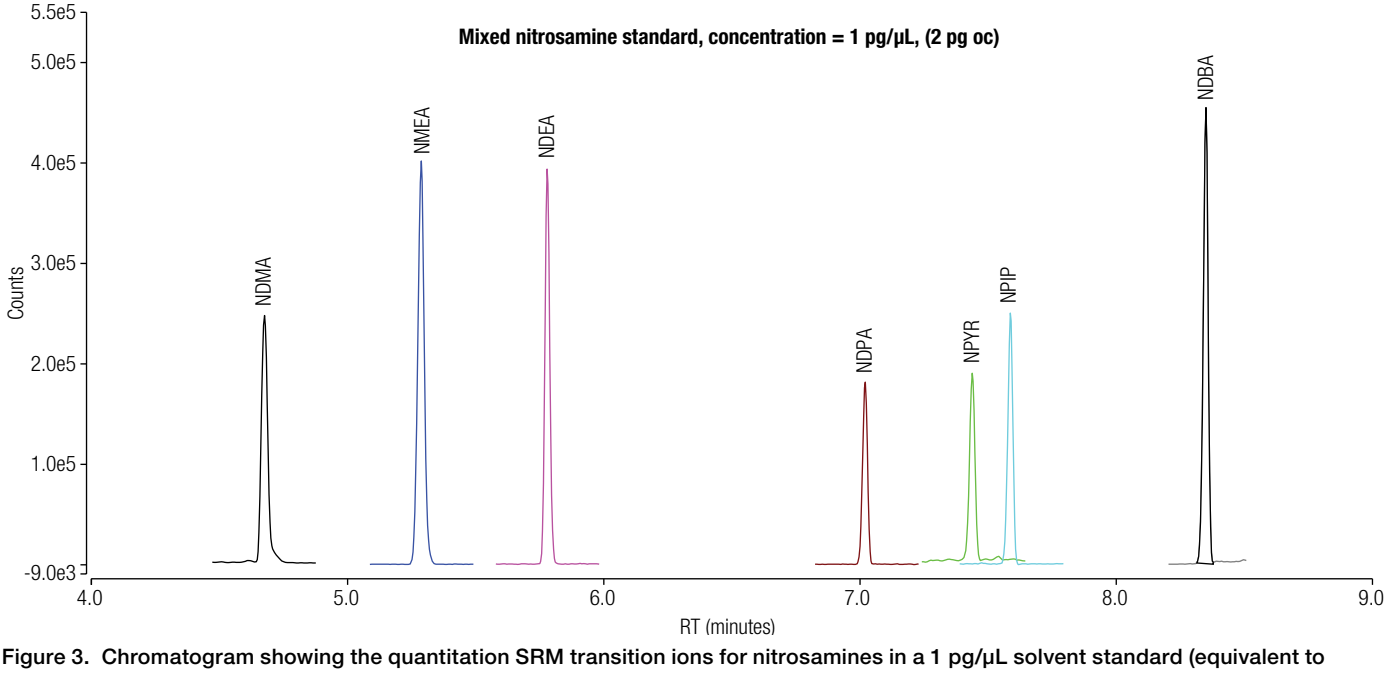

Figure 3. Chromatogram showing the quantitation SRM transition ions for nitrosamines in a 1 pg/µL solvent standard (equivalent to 1 ng/L in sample) with excellent chromatographic peak shapes for all compounds. (NDMA-d6 was not displayed to show peak shape for NDMA).

Sensitivity

The enhanced sensitivity of the new AEI source is demonstrated for NDMA in Figure 4. Here a 0.01 pg/µL (0.02 pg oc) solvent standard shows excellent signal precision with peak area repeatability <10% RSD at low ppt levels (equivalent to low ppq [0.01 ng/L] in sample extracts).

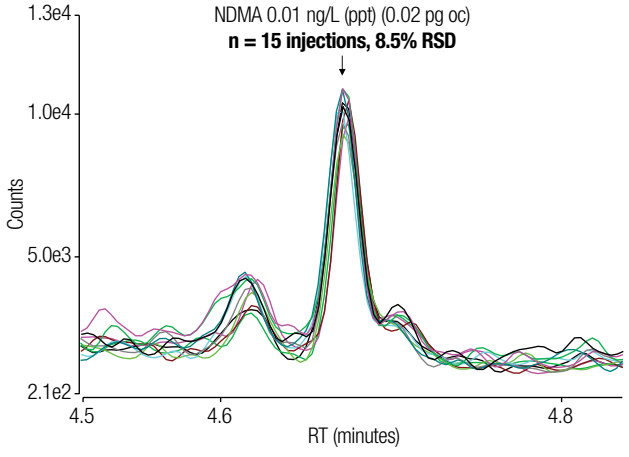

Figure 4. Overlaid quantification SRM transitions (74 → 44 *m/z*) from n=15 consecutive injections of a 0.01 pg/µL NDMA solvent standard corresponding to 0.01 ng/L in sample. No data smoothing was used and data was acquired in timed-SRM mode.

To assess the instrument detection limit (IDL), 15 consecutive injections were performed using the 0.01 and 0.1 pg/µL solvent standards. The IDL for each individual compound was then calculated by taking into account the on-column amount, % RSD of peak area repeatability from n=15 injections, and *t*-score of 2.624, corresponding to 14 degrees of freedom at 99% confidence (Table 2).

Table 2. Calculated instrument detection limit (IDLs) and absolute peak area repeatability (as % RSD) for nitrosamines determined from n=15 injections of either a 0.01 pg/µL or 0.1 pg/µL solvent standards where the peak area % RSD was lower than 15%

	Calculated IDL values		
Component	Concentration injected (pg oc*)	Peak area % RSD	IDL (pg oc*) equivalent to ng/L in sample
NDMA	0.02	8.5	0.005
NMEA	0.02	5.2	0.003
NDEA	0.02	7.9	0.004
NDPA	0.20	7.7	0.040
NPYR	0.20	10.9	0.060
NPIP	0.02	12.0	0.006
NDBA	0.02	9.9	0.005

*oc = on column, *t*-score = 2.624, n=14 degrees of freedom

Linearity

Nitrosamines linearity was determined using dichloromethane solvent standards at concentrations ranging from 0.05 to 20 pg/µL (corresponding to 0.05–20 ng/L in water extracts). Linear regression curves were plotted as average values of n=3 injections per calibration level. All compounds showed excellent linear response with coefficient of determination $R^2 > 0.999$, and average response factor values (RF % RSD) across this concentration range < 5% (Figure 5).

Method Detection Limit (MDL) determination The method detection limit was derived in the same way as for the solvent standard derived IDL except that 1 L ultra-pure water was fortified with nitrosamines prior to extraction at 0.1 and 0.5 ng/L. Excellent limits of detection were demonstrated down to low ppq levels in sample. The results for the method detection limit are outlined below with values ranging from 0.008 to 0.045 ng/L (Table 3).

Calculated LOQ in sample

The LOQ was determined as the lowest concentration of nitrosamines passing the following criteria:

- Ion ratios within ±30% of the expected values calculated as an average across a calibration curve ranging from 0.05 to 100 pg/µL (corresponding to 0.05–100 ng/L in drinking water)
- Measured ion ratio % RSD < 15%
- Ion co-elution within ±0.01 minutes
- Peak area repeatability of < 15% RSD

To demonstrate the method LOQs, water was fortified with nitrosamines prior to extraction at 0.1 and 0.5 pg/µL. These were injected 10 times, and based on satisfaction of criteria above, the LOQs for compounds ranged from 0.1 to 0.5 ng/L (Table 4).

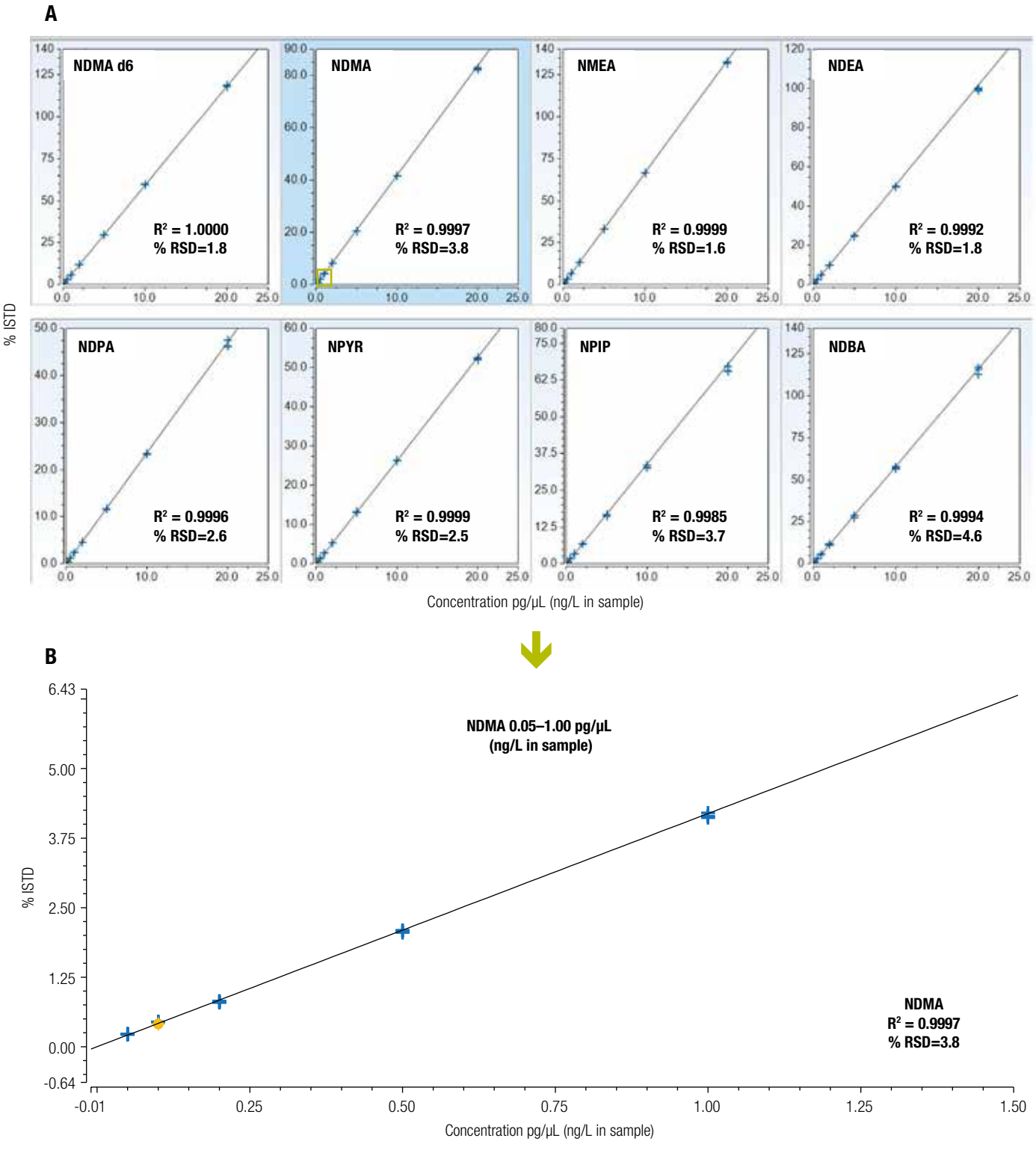

Figure 5. (A) Linearity of targeted compounds demonstrated using a solvent-based calibration curve ranging from 0.05 to 20 pg/µL (corresponding to 5–20 ng/L in drinking water). Average calibration factor (AvCF) function was used in Chromeleon CDS software with three replicate injections at each concentration and internal standard adjustment was conducted using NDPA d-14. Coefficient of determination (R^2) and average response factor values (RF % RSD) are displayed. (B) Expanded region of calibration for NDMA from 0.05–1.00 pg/µL (corresponding to 0.05–1.00 ng/L in drinking water) showing excellent precision for triplicate injections per point.

Table 3. Calculated method detection limit (MDLs) and absolute peak area repeatability (as % RSD) for nitrosamines determined from n=10 injections of water fortified with nitrosamines prior to extraction at 0.1 and 0.5 ng/L.

Component	Calculated MDL values		
	Concentration injected (pg oc*)	Peak area % RSD	IDL (pg oc*) equivalent to ng/L in sample
NDMA	0.2	1.5	0.03
NMEA	0.2	3.1	0.01
NDEA	0.2	3.4	0.01
NDPA	1.0	4.0	0.02
NPYR	1.0	3.8	0.02
NPIP	0.2	4.9	0.05
NDBA	0.2	1.6	0.01

*oc = on column, *t*-score = 2.821, n=9 degrees of freedom, 99% confidence level, peak area % RSD < 15%

Table 4. Method LOQ values derived for nitrosamines in drinking water from injecting n=10 times 0.1 ng/L and 0.5 ng/L fortified water extracts. The criteria used to assess individual nitrosamine LOQ values were ion ratio % deviation from theoretical, measured ion ratio % RSD, peak area % RSD, and ion coelution.

Component	RT	Conc. injected (pg oc*)	Target ion ratio** %	Mean measured % ion ratio	Measured ion ratio % RSD	Mean ion ratio abundance % deviation	Pass criteria	Peak area % RSD	Pass criteria	LOQ (ng/L)
NDMA	4.8	0.2	164	154	6.6	6.9	±30%	1.5	<15%	0.1
NMEA	5.5	0.2	50	50	9.5	8.1	±30%	3.1	<15%	0.1
NDEA	6.0	0.2	33	34	6.2	5.0	±30%	3.4	<15%	0.1
NDPA	7.2	1.0	35	33	4.8	5.5	±30%	4.0	<15%	0.5
NPYR	7.6	1.0	37	41	9.4	13.3	±30%	3.8	<15%	0.5
NPIP	7.8	0.2	91	94	10.6	9.7	±30%	4.9	<15%	0.1
NDBA	8.5	0.2	21	21	1.7	1.5	±30%	1.6	<15%	0.1

*oc = on column, **derived from average ion ratio across calibration range 0.05-20 ng/L, n=10 injections of tap water spiked at 0.1 ng/L pre-extraction, *t*-score= 2.821, n=9 degrees of freedom. peak area % RSD <15%, criteria for ion coelution ±0.01 min deviation

Due to the unrivaled sensitivity and selectivity of the new TSQ 9000 AEI GC-MS/MS system, accurate quantitation of nitrosamines down to low ppq (ng/L) levels in sample is now achievable. The chromatograms for individual nitrosamines at the relevant LOQ in extracted water are shown with confirmed qualifier within ±15% (Figure 6).

Method accuracy

The method performance was assessed by evaluating the compound recoveries determined from three separate extractions of a 50 ng/L nitrosamine fortified water sample. The results show that the average recovery values ranged between 80.7% and 111.1% (Table 5). This was comfortably within the 70–130% criteria set for this method, showing that the extraction procedure had excellent recovery for nitrosamines in drinking water.

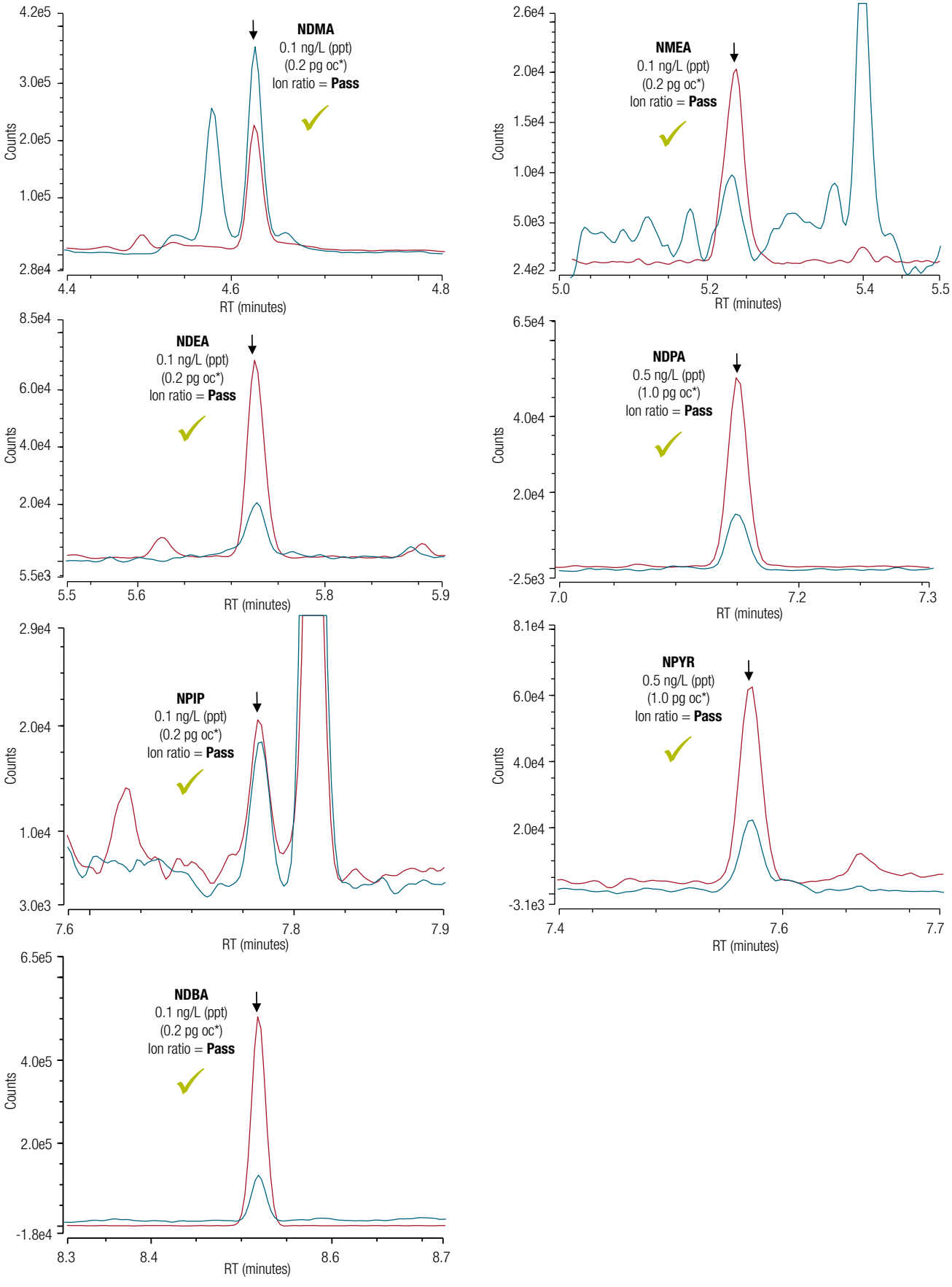

Figure 6. Individual chromatograms of nitrosamines with overlaid quantitation and qualifier ions at the LOQ in sample at between 0.1 ng/L and 0.5 ng/L in ultra-pure water. All the qualification ion ratios were found to be within ±15% of the average value calculated across the range of calibration standards 0.05 to 100 pg/µL (corresponding to 0.05–100 ng/L in drinking water).

Table 5. % Recovery determined from three separate nitrosamine fortified water extractions at 50 ng/L. NDMA d-6 and NDEA d-10 surrogate standards were spiked into 1 L of water at 25 ng/L to correct recoveries for NDMA and NDEA.

Compound	RT (min)	Concentration (ng/L)	Calculated (ng/L)	% Recovery	Pass/ Fail	Limits Recovery %
NDMA	4.7	50.0	54.2	108.4	PASS	70–130
NMEA	5.3		41.5	83.0	PASS	
NDEA	5.8		55.5	111.1	PASS	
NDPA	7.0		40.4	80.7	PASS	
NPYR	7.4		48.3	96.5	PASS	
NPIP	7.6		45.0	90.0	PASS	
NDBA	8.4		42.2	84.3	PASS	

Quantification of nitrosamines in drinking water samples

Seventeen drinking water samples were obtained from water testing facilities across Europe and the total nitrosamine content was quantified as total nitrosamines in ng/L, taking into account any nitrosamine present above the LOQ (as defined in Table 4). All drinking water samples contained nitrosamines with values ranging between 0.9 and 4.5 ng/L (Figure 7). Out of the nitrosamines present in drinking water, NDMA, NDBA, and NDEA were the most prevalent with calculated NDMA amounts ranging from 0.2 to 3.5 ng/L. For all of the samples, the amount of nitrosamines was below the threshold value of 10 ng/L.[3,4] This demonstrates that the TSQ 9000 AEI GC-MS/MS system is capable of detecting and quantifying nitrosamines in drinking water easily down to sub ppt levels, and if regulation arises, is ideally positioned for this type of analysis.

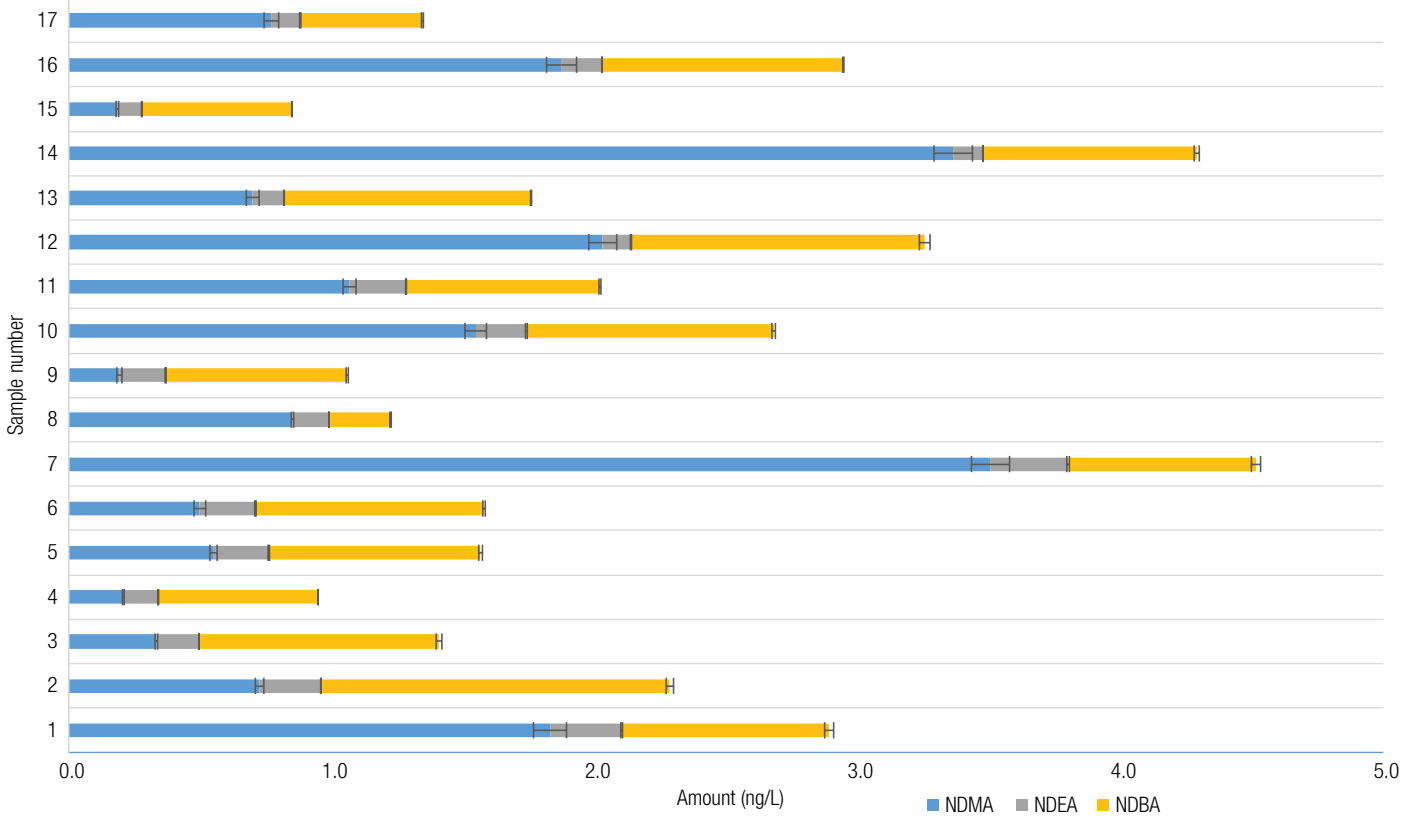

Figure 7. Total quantified nitrosamine content (ng/L) from 17 drinking water samples sourced from separate water testing facilities across Europe. NDMA d-6 and NDEA d-10 surrogate standards were spiked to 1 L of water pre-extraction at 25 ng/L to correct recoveries for NDMA and NDEA. Deuterated NDBA was not available for the analysis so the values are not corrected. The mean and standard deviation for triplicate injections per sample are presented in the chart.

Conclusions

The results of the experiments described here demonstrate:

- Excellent sensitivity with unrivaled instrument detection limits for nitrosamines in solvent standards down to low ppt levels 0.003 pg oc translating to 0.003 ng/L (low ppq w/v) in sample.
- Outstanding linearity used for the quantification of nitrosamines in 17 drinking water samples analyzed was demonstrated over a range of 0.05 to 20 pg/µL (corresponding to 0.05–20 ng/L (ppt w/v) in drinking water). All compounds showed excellent linear responses with coefficient of determinations $R^2 > 0.999$ and average response factor % RSDs < 5%.
- The MDL for nitrosamines was calculated to be between 0.008 and 0.045 ng/L (ppt w/v).
- The LOQ for the method was set at between 0.1 and 0.5 ng/L (ppt w/v) for nitrosamines in drinking water with data from n=10 injections of LOQ standard, having ion ratio % deviation from the average of the calibration standards within ±15%, peak area % RSD < 15%, and ion co-elution within 0.01 minutes.
- Compound recoveries were found to be between 80.7% and 111.1%, well within the set method performance limits of 70–130%.
- Seventeen drinking water samples from separate water testing facilities across Europe were quantified and total nitrosamine content ranged between 0.9 and 4.5 ng/L.

Taken together these results demonstrate that the TSQ 9000 GC-MS/MS system configured with the AEI source provides unparalleled levels of quantitative performance making it an ideal analytical tool for routine laboratories.

Appendix. SRM transitions

Name	RT (min)	(SRM) *m/z*		
		Mass (*m/z*)	Product Mass (*m/z*)	Collision energy V
NDMA-d6	4.7	80	50	5
		80	46	15
NDMA	4.8	74	42	15
		74	44	5
NMEA	5.5	88	71	5
		88	42	15
NDEA-d10	5.9	112	34	5
		112	50	10
NDEA	6.0	102	85	5
		102	44	10
NDPA-d14	7.1	78	46	10
		110	78	5
NDPA	7.2	130	113	5
		130	43	10
NPYR	7.6	100	55	5
		100	70	5
NPIP	7.8	114	84	5
		114	97	5
NDBA	8.5	116	99	5
		158	99	5

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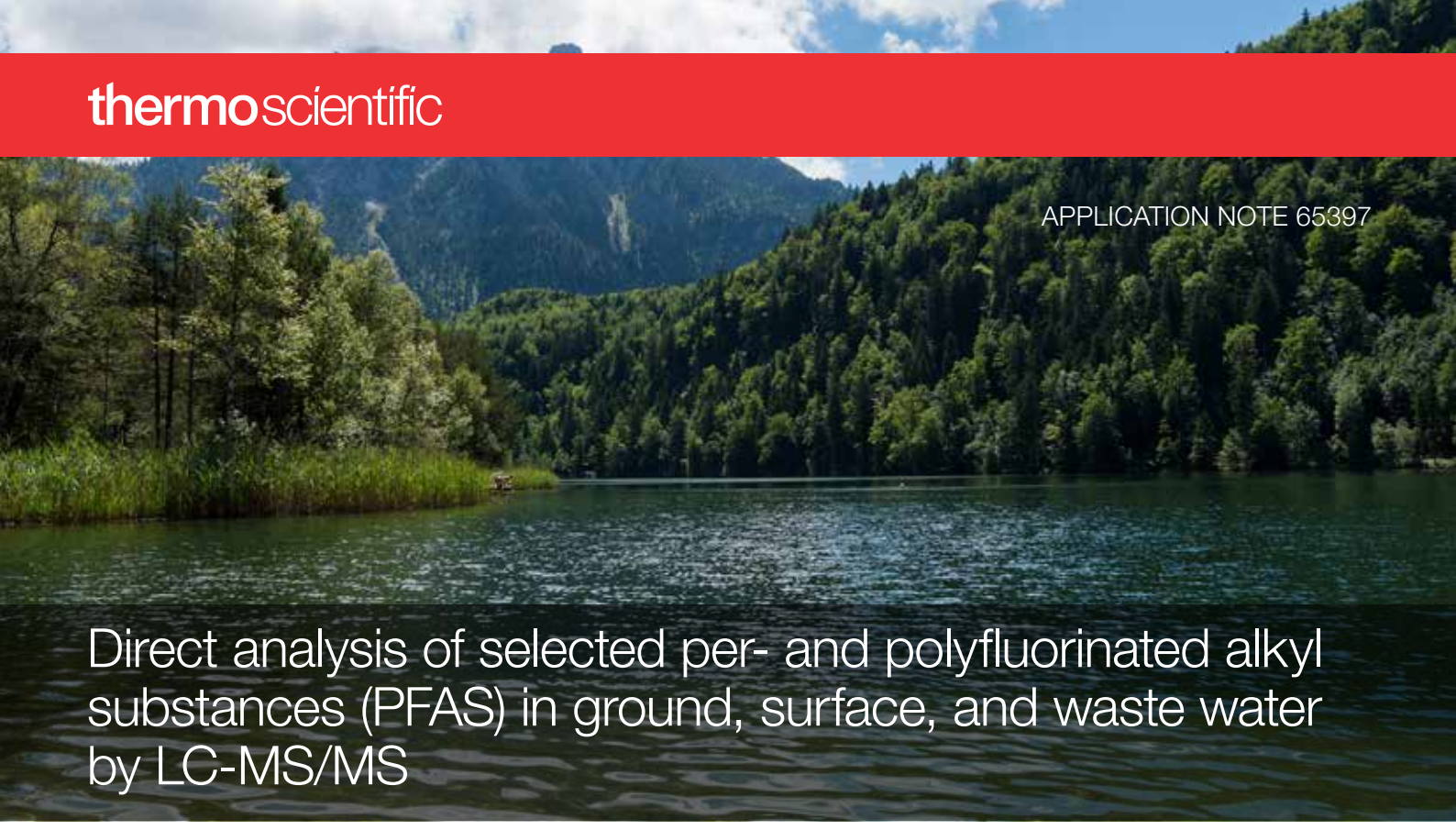

APPLICATION NOTE 65397

Direct analysis of selected per- and polyfluorinated alkyl substances (PFAS) in ground, surface, and waste water by LC-MS/MS

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Keywords

Perfluorinated organic compounds, PFAA, PFOS, PFOA, GenX, PFCs, environmental contaminants, emerging contaminants, EPA 8327, EPA 537, EPA 537.1

Goal

To demonstrate method performance for the PFAS analysis at low levels (ng/L) in a wide variety of non-drinking water matrices by direct analysis and submit data package for EPA 8327 interlaboratory method validation.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that includes perfluorooctanoic (PFOA), perfluorooctyl sulfonic acid (PFOS), and hexafluoropropylene oxide dimer acid (HFPO-DA, which is part of GenX process). PFAS compounds have been manufactured since the 1940s. The most well-known PFAS compounds, PFOA and PFOS, have been the most extensively produced and studied for chemical properties and toxicological effects. Both chemicals are very persistent in the environment and accumulate in the human body over time. It is well documented that exposure to PFAS can lead to adverse human health effects[1-3] and are found in food packaging material as well as food processing equipment. Plants can accumulate PFAS when grown in PFAS-containing soil and/or water. These compounds are also found in a wide variety of consumer products such as

cookware, food containers (e.g., pizza boxes), and stain repellants. Additional products that lead to routes of exposure include clothing with stain- and water-repellent fabrics, nonstick products (e.g., Teflon), polishes, waxes, paints, and cleaning products. Another major source of PFAS are fire-fighting foams, which are a primary component of groundwater contamination at airports and military bases. More exposure comes from workplace environments, including production facilities or industries (e.g., chrome plating, electronics and manufacturing, or oil recovery).

Of particular note, drinking water can contain PFAS and can be associated with domestic and specific workplace facilities. Living organisms, including fish, animals and humans, have been shown to have accumulations of PFAS compounds and thus can build up and persist over time.[1-4] For these reasons, most people have been exposed to PFAS.

There is documented evidence that exposure to PFAS can lead to adverse health outcomes in humans.[3,4] Many studies indicate that PFOA and PFOS can cause reproductive and developmental, liver and kidney, and immunological effects in laboratory animals. Both chemicals have been found to cause tumors in animals. The most consistent findings are increased cholesterol levels among exposed populations, with more limited findings related to the following:

- low infant birth weights
- effects on the immune system
- cancer (for PFOA)
- thyroid hormone disruption (for PFOS)

PFAS compounds can be per- and polyfluorinated along a carbon backbone, typically ending with a carboxylic or sulfonic acid. PFOA and PFOS are made up of a C_8F_{17} subunit with either a carboxylic group (PFOA) or sulfonate group (PFOS). Replacement chemicals, like GenX, tend to have fewer carbon atoms in the chain, but have many similar physical and chemical properties as their predecessors (e.g., they both repel oil and water). Industries in the United States have phased out production of PFOA and PFOS because of health risks to humans and have been using replacement PFAS, such as GenX. There is a substantial body of knowledge for managing risk from PFOS and PFOA, but much less knowledge about the replacement PFAS.

The US EPA office of Ground Water and Drinking Water has developed a method specifically for the analysis of PFAS in drinking water, EPA 537, which is based on solid-phase extraction (SPE) followed by LC-MS/MS detection.[5] This methodology was developed for use during the EPA's Unregulated Contaminant Rule 3 (UCMR3) monitoring program.[6] Recently, an updated version of this method EPA 537.1 has been validated to include additional PFAS compounds such as GenX.[8] An alternative method developed for additional water matrices such as surface, ground, and waste waters is ASTM D7979,[7] and is based on simple sample extraction and filtration followed by LC-MS/MS analysis. This application note describes a direct analysis method for the determination of a list of 24 PFAS in a wide variety of non-drinking water matrices. The data was used for the validation of a new method, EPA 8327, for a wide variety of water matrices as part of an interlaboratory study sponsored by the EPA Office of Water.

Experimental

This application note describes the quantitation of selected PFAS in reagent, ground, surface, and waste water based on the recent EPA 8327 method. The list of PFAS included in this study is shown in Table 1.

LC-MS/MS analysis

Since the required limits of detection are in the low ng/L range, careful selection of reagents and consumables is necessary to ensure they are PFAS-free. Therefore, the LC-MS/MS system comprised a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system fitted with a Thermo Scientific™ PFC-free kit (P/N 80100-62142) and interfaced with a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer equipped with a HESI ionization probe. An isolator column was also installed after the LC pump and prior to the injection valve to offset background contaminants from the LC pump, autosampler, degasser, and mobile phases.

Table 1. List of PFAS compounds included in this method

Analytes	Abbreviation	CAS number	Surrogates
PFAS Sulfonic Acids			
Perfluorobutyl sulfonic acid	PFBS	29420-49-3	$^{13}C_3$-PFBS
Perfluorohexyl sulfonic acid	PFHxS	3871-99-6	$^{13}C_3$-PFxS
Perfluorooctyl sulfonic acid	PFOS	1763-23-1	$^{13}C_8$-PFOS
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid	4:2 FTS	757124-72-4	$^{13}C_2$-4:2 FTS
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	$^{13}C_2$-6:2 FTS
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid	8:2 FTS	39108-34-4	$^{13}C_2$-8:2 FTS
Perfluoro-1-pentanesulfonic acid	PFPeS	706-91-4	-
Perfluoro-1-heptanesulfonic acid	PFHpS	375-92-8	-
Perfluoro-1-nonanesulfonic acid	PFNS	68259-12-1	-
Perfluoro-1-decanesulfonic acid	PFDS	2806-15-7	-
PFAS Carboxylic Acids			
Perfluorobutanoic acid	PFBA	375-22-4	$^{13}C_4$-PFBA
Perfluoropentanoic acid	PFPeA	2706-90-3	$^{13}C_5$-PFPeA
Perfluorohexanoic acid	PFHxA	307-24-4	$^{13}C_5$-PFHxA
Perfluoroheptanoic acid	PFHpA	375-85-9	$^{13}C_4$-PFHpA
Perfluorooctanoic acid	PFOA	335-67-1	$^{13}C_8$-PFOA
Perfluorononanoic acid	PFNA	375-95-1	$^{13}C_9$-PFNA
Perfluorodecanoic acid	PFDA	335-76-2	$^{13}C_6$-PFDA
Perfluoroundecanoic acid	PFUnA	2058-94-8	$^{13}C_7$-PFUnA
Perfluorododecanoic acid	PFDoA	307-55-1	$^{13}C_2$-PFDoA
Perfluorotridecanoic acid	PFTriA	72629-94-8	-
Perfluorotetradecanoic acid	PFTreA	376-06-7	$^{13}C_2$-PFTreA
PFAS sulfonamides and sulfonamidoacetic acids			
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	2991-50-6	D_3-N-EtFOSAA
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	D_3-N-MeFOSAA
Perfluoro-1-octanesulfonamide	PFOSA	754-91-6	$^{13}C_8$-PFOSA

LC conditions	
Analytical column:	Thermo Scientific™ Accucore™ RP-MS, 2.6 µm, 2.1 × 100 mm (P/N 17626-102130)
Isolator column:	Thermo Scientific™ Hypersil™ BDS C18, 5 µm, 2.1 × 50 mm (P/N 28105-052130)
Column temp.:	45 °C
Flow rate:	0.5 mL/min
Solvent A:	Water containing 2 mM ammonium acetate, 2% methanol, and 0.1% acetic acid
Solvent B:	Methanol containing 2 mM ammonium acetate, 2% water, and 0.1% acetic acid

LC conditions (continued)		
Injection volume:	25 µL	
Gradient:	Time (min)	% Solvent B
	0	0
	1	30
	6	45
	13	80
	14	95
	17	95
	18	0
	21	0

Optimized MS parameters	
HESI source:	Negative ionization mode
Spray voltage:	2.5 kV
Sheath gas:	50 arb
Auxiliary gas:	10 arb
Ion transfer tube temp.:	325 °C
Vaporizer temperature:	300 °C

Optimized MS parameters (continued)	
Cycle time for the negative SRM transitions:	0.3 s
Q1 resolution:	0.7 Da
Q3 resolution:	1.2 Da
CID gas:	2 mTorr

Table 2 summarizes the monitored SRM transitions.

Table 2 (part 1). Monitored SRM transitions details

Compound	Retention time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
PFBA	2.70	212.979	168.97	9	30
$^{13}C_4$-PFBA	2.70	216.993	172	9	30
PFPeA	4.98	262.976	219.042	9	31
$^{13}C_5$-PFPeA	4.98	267.993	222.99	9	32
PFBS	5.73	298.943	79.957	34	116
		298.943	98.956	29	116
$^{13}C_3$-PFBS	5.73	301.953	79.96	34	119
PFHxA	7.94	312.973	119.042	18.76	39
			268.97	9	39
$^{13}C_5$-PFHxA	7.94	317.99	273	9	37
4:2 FTS	7.66	326.974	81.042	26.07	115
			286.958	23	115
			307.042	18.11	115
$^{13}C_2$-4:2 FTS	7.66	328.981	308.96	18	103
PFPeS	8.42	348.94	80.042	33.66	145
			99	31	145
			119.054	31.42	145
PFHpA	9.91	362.97	119.054	19.52	43
			168.97	15.53	43
			319.042	9	43
$^{13}C_4$-PFHpA	9.91	366.983	321.98	9	43
PFHxS	10.11	398.937	79.957	39	135
			98.956	35	135
$^{13}C_3$-PFxS	10.11	401.947	79.957	39	133
PFOA	11.22	412.966	169	16.1	49
			219	14.55	49
			369.042	9	49
$^{13}C_8$-PFOA	11.22	420.993	376	9	48
6:2 FTS	11.12	426.968	81.042	29.94	123
			386.97	26.72	123
			406.988	21.45	123
$^{13}C_2$-6:2 FTS	11.12	428.975	408.96	21	123
PFHpS	11.30	448.933	80.012	37.6	131
			98.97	36.2	131
			169.03	31.04	131

Table 2 (part 2). Monitored SRM transitions details

Compound	Retention time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
PFNA	12.21	462.963	169	17.51	52
			219.012	15.23	52
			418.97	9	52
$^{13}C_9$-PFNA	12.21	471.993	426.97	9	52
PFOS	12.24	498.93	79.957	47	159
			98.956	40	159
$^{13}C_8$-PFOS	12.24	506.957	79.957	40	160
PFDA	11.58	512.96	219.012	16.14	56
			269.042	15.8	56
			469.042	9	56
$^{13}C_6$-PFDA	11.58	518.98	473.97	9	56
8:2 FTS	13	526.962	81.012	34.83	137
			487	28.92	137
			506.97	24.37	137
$^{13}C_2$-8:2FTS	13	528.968	508.96	24	137
PFNS	13.04	548.927	80.071	42.34	148
			98.97	40.67	148
			229.958	41.66	148
PFUdA	13.73	562.957	219	17.32	62
			269.03	16.94	62
			518.97	9	62
NMeFOSAA	13.64	569.967	418.97	18.42	107
			512	19.55	107
$^{13}C_7$-PFUnA	13.73	569.98	524.97	9	62
d_3-N-MeFOSAA	13.64	572.986	418.97	18	107
PFOSA	13.66	497.946	78.071	29.37	127
			169.03	25.85	127
			478.042	22.51	127
$^{13}C_8$-PFOSA	13.66	505.973	77.97	29	127
NEtFOSAA	14.04	583.983	418.97	18.34	101
			482.958	13.9	101
			526.03	18.26	101
d_5-N-EtFOSAA	14.04	589.014	418.97	18	101
PFDS	13.70	598.924	80.042	44.92	169
			98.929	43.48	169
			229.929	46.09	169
PFDoA	14.30	612.954	169.03	23.69	67
			319.042	17.54	67
			569	9	67
$^{13}C_2$-PFDoA	14.30	614.96	569.97	9	67
PFTriA	14.63	662.95	168.97	25.16	71
			369.071	17.85	71
			619.042	9	71
PFTreA	14.83	712.947	319.054	19.86	74
			369.042	18.87	74
			668.97	9	74
$^{13}C_2$-PFTreA	14.83	714.954	669.96	9	74

Data processing

Thermo Scientific™ Chromeleon™ Chromatography Data System software, version 7.2.9

All materials were demonstrated to be free from interferences by analyzing method blanks. All glassware, including syringes and filters, were thoroughly cleaned with methanol prior to sample preparation. All solvents used in sample preparation, standards preparation, and chromatography were Thermo Scientific UHPLC-MS grade.

Sample preparation

PFAS standard solutions

Target and surrogate PFAS standard mixtures in methanol at 2000 and 1000 µg/L, respectively, were purchased from Wellington Laboratories and kept away from PFAS packaging and material during storage. A stock solution of 24 target PFAS compounds was prepared in methanol at a concentration of 2 µg/L. Calibration solutions, with concentrations of 5–200 ng/L (ppt), were prepared by serial dilutions of the stock solution in 50:50 (v/v) methanol/water containing 0.1% acetic acid.

Non-drinking water matrices

Field water samples (5 mL) were provided by the US EPA Region 5 and included reagent water, surface water, ground water, and waste water through a participating EPA study. Each water sample was spiked with a low (60 ng/L) and high level (200 ng/L) of a selected target PFAS compounds (five replicates of each) prior to shipment to the lab. Five blank samples of each water matrix were also provided.

The 5 mL water samples were then spiked with 40 µL of a 20 µg/L isotopically labeled PFAS surrogates solution (Table 1). 5 mL of methanol were added and the mixture vortexed for 1 minute. The mixture was then filtered through a washed Acrodisc® GxF/0.2 µm GHP membrane syringe-driven filter with methanol and acetonitrile (Pall Corporation, P/N AP-4305). The 10 mL filtrates were acidified by addition of 10 µL of acetic acid, and an aliquot of each sample was transferred to a polypropylene autosampler vial (Thermo Fisher Scientific, P/N C4013-13) sealed with a polyethylene cap with integrated polyethylene membrane (P/N C4013-50Y).

Control samples

The EPA 8237 method requires control samples (method blank, laboratory control, and reporting limit checking samples) to be run with field non-drinking water samples. Therefore, two method blanks were prepared by measuring 5 mL of water UHPLC-MS grade into 15 mL polypropylene Falcon™ tubes (BD Falcon, P/N 14-959-70C) and spiking with 40 µL of a 20 µg/L PFAS surrogate solution in methanol. Two laboratory control samples were prepared by spiking 5 mL of water UHPLC-MS grade at 160 ng/L of 24 selected PFAS, and a reporting limit of quantitation checking sample was prepared by spiking 5 mL of water UHPLC-MS grade at 10 ng/L. Control samples were then taken through the sample preparation as field water samples.

Results and discussion

Excellent chromatographic separation was achieved on an Accucore RP-MS analytical column using different mobile phases compositions. Figure 1 shows an overlaid chromatogram of all PFAS compounds analyzed in this method.

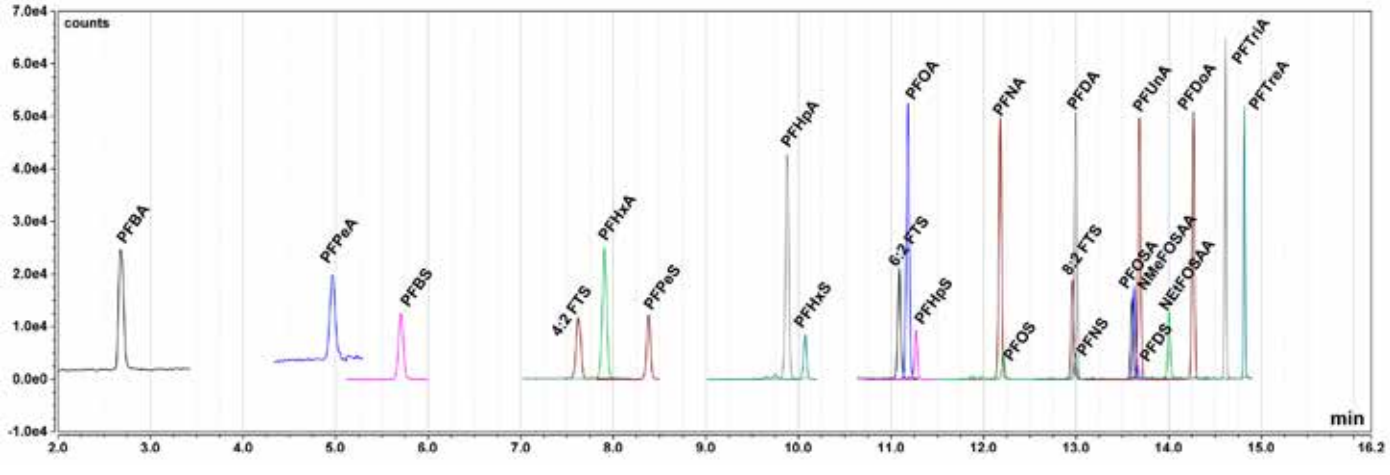

Figure 1. Overlaid chromatograms of all PFAS compounds included in this method

Linearity and sensitivity

Excellent linearity and quantitative accuracy were achieved over the range of 5 to 200 ng/L, with correlation coefficients greater than 0.99 for all transitions and the respective residuals within 20% of the nominal values. Representative calibration curves for PFOS and PFTriA are shown in Figure 2, with correlation coefficients of 0.9955 and 0.9950, respectively. Figure 2 also shows chromatograms of overlaid quantitation and confirming ions injected at 1 ng/L, which is five times lower than the LLOQ reported by ASTM D7979-17 for these two compounds. Additionally, Table 3 shows the LLOQs for all 24 PFAS analyzed in this method, based on accuracy and RSD ≤20%, demonstrating the high sensitivity achieved with the TSQ Altis mass spectrometer for the quantitation of PFAS at very low levels (ppt range).

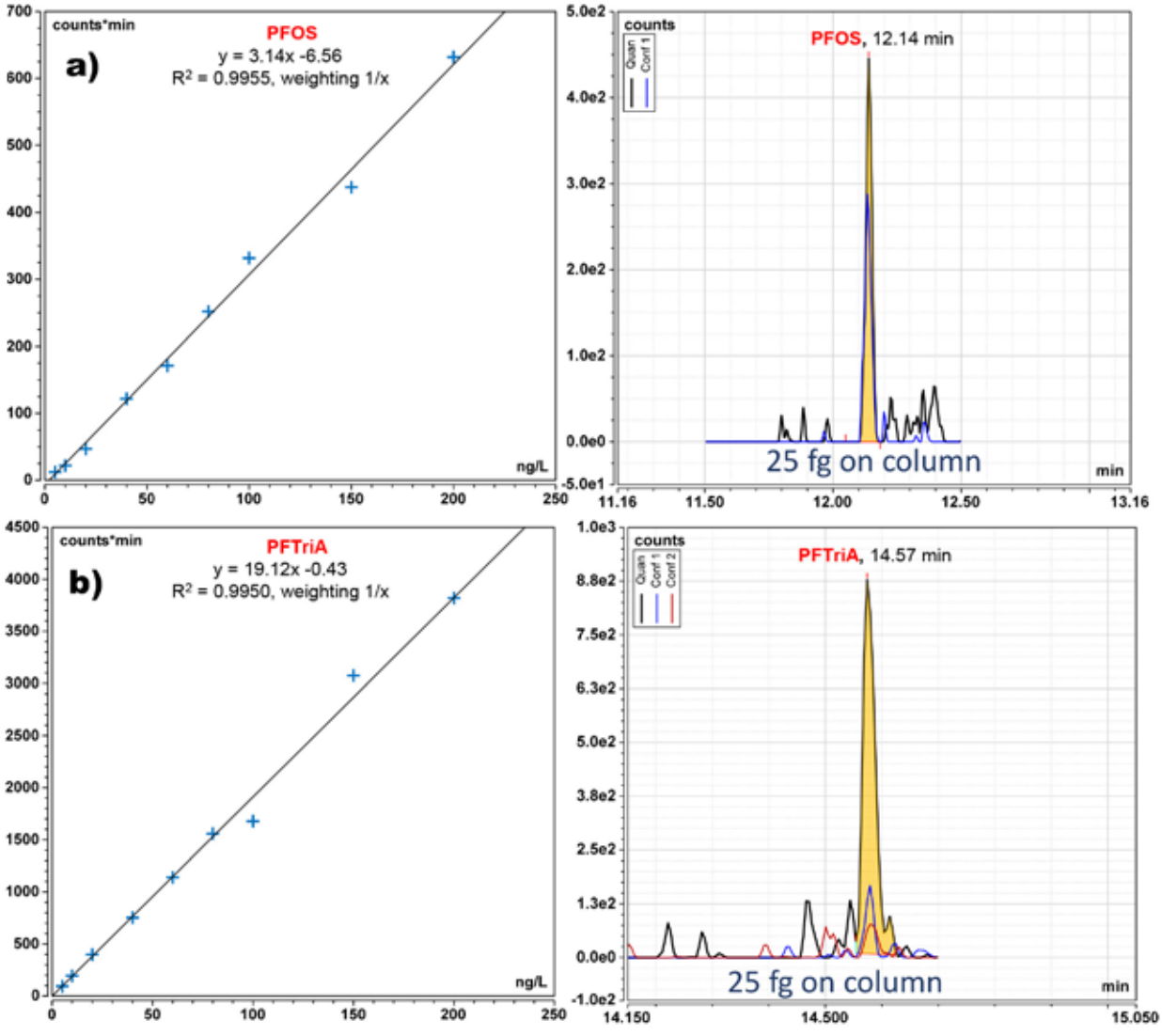

Figure 2. Representative calibration curves for a) PFOS and b) PFTriA, and chromatograms of an injection of 1 ng/L, which is five times lower than the reporting limit of quantitation

Table 3. Reporting lower limit of quantitation obtained by this method and ASTM D7979-17 reporting ranges

Compound	LLOQ* (N=3) (ng/L)	ASTM D7979-17 reporting ranges* (ng/L)
PFBA	10	50–2000
PFPeA	10	50–2000
PFBS	2	10–400
4:2 FTS	10	-
PFHxA	2	10–400
PFPeS	2	-
PFHpA	5	10–400
PFHxS	5	10–400
6:2 FTS	5	-
PFOA	2	10–400
PFHpS	2	-
PFNA	2	10–400
PFOS	2	10–400
8:2 FTS	5	-
PFDA	2	10–400
PFNS	10	-
N-MeFOSAA	5	-
PFOSA	10	-
PFDS	10	-
PFUnA	2	10–400
N-EtFOSAA	5	-
PFDoA	2	10–400
PFTriA	2	10–400
PFTreA	2	10–400

*Concentrations taking into consideration the 50% dilution with methanol.

Control samples

Table 4 summarizes the method control criteria, and the results demonstrate all compounds passed in this method. Figure 3 shows the overlaid chromatogram of all PFAS of a method blank and a reagent water spiked at 10 ng/L (LLOQ checking sample) and taken through sample preparation. PFBA and PFPeA are quantifiable at an injected concentration of 5 ng/L, which is much lower than the reported limit of quantitation in EPA 8327 and ASTM D7979 (25 ng/L without considering 2-fold dilution in methanol).

Sample analysis

Each water matrix was spiked at low and high concentrations as described, (N=5 ea.) The 60 samples received were divided into three batches of 20 samples and analyzed on three different days. All 24 PFAS compounds were detected and quantifiable at both low and high spike concentrations. Figure 4 shows an example of overlaid chromatograms of all PFAS spiked at 60 ng/L in reagent, ground, surface, and waste samples. In Figure 4 fronting was observed with the first eluting chromatographic peaks in ground, surface, and waste water samples due to the overload of the analytical column by large injection volumes (25 µL). Reduced injection volumes (15 µL) improved peak shape and will also improve robustness (due to less matrix on column) while still maintaining good sensitivity as shown in Figure 5.

Table 4. Summary of method control criteria

Sample Type	Definition	Criteria	Results
Reagent blank	Methanol: Water (50:50, v/v) + 0.1% acetic acid	Concentration must be one half the LLOQ	Target compounds NOT DETECTED OR BELOW <LLOQ
Method blank	Reagent water + surrogates at 160 ng/L. Taken through sample preparation	Concentration must be one half the LLOQ	Target compounds NOT DETECTED OR BELOW LLOQ
LLOQ checking	Reagent water + targets at 10 ng/L. Taken through sample preparation	S/N ratio ≥3 for all quantitative ions & Target Recoveries <50% deviation	LLOQ at 10 ppt Recoveries <30% deviation for most of the compounds
Laboratory controls	Reagent water + targets at 160 ng/L. Taken through sample preparation.	Target recoveries <30% deviation	Target recoveries <30% deviation for most of the compounds

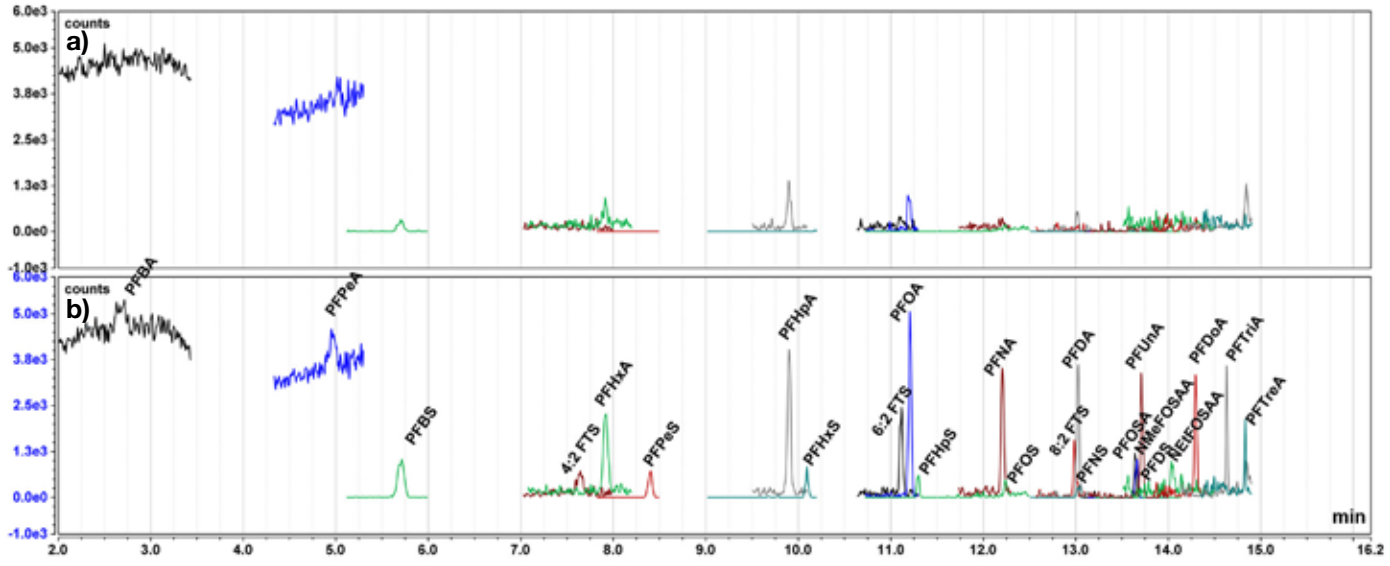

Figure 3. PFAS overlaid chromatograms: a) method blank sample and b) reporting limit checking sample spiked at 10 ng/L

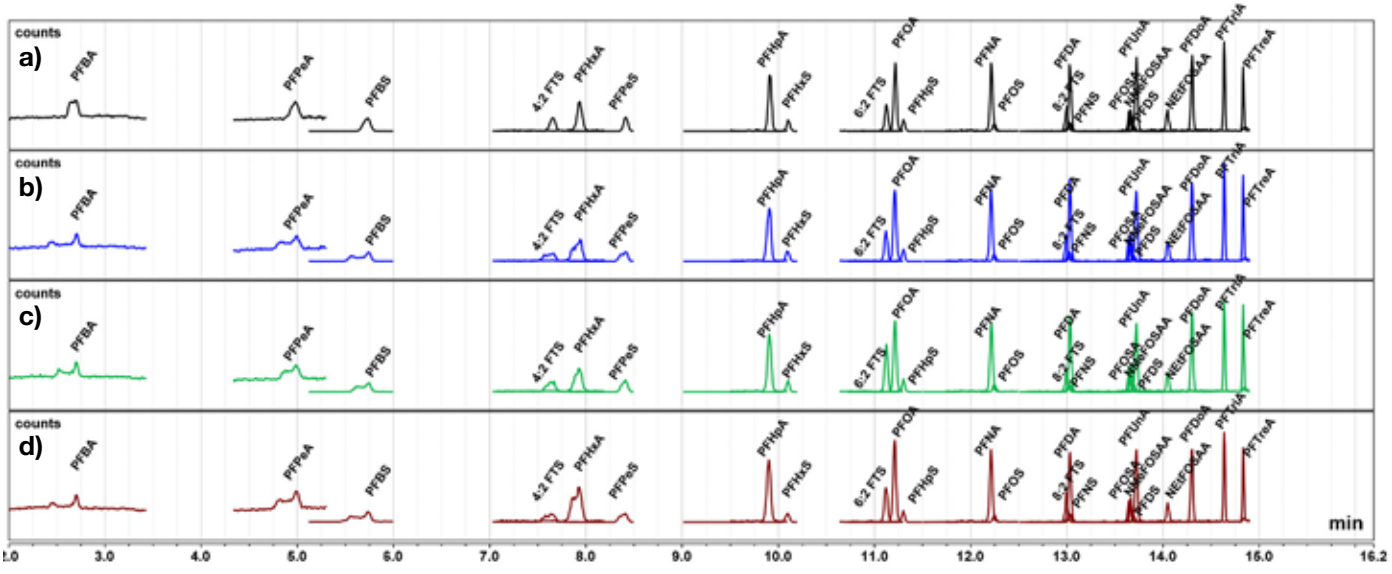

Figure 4. Overlaid chromatograms of 24 PFAS spiked at 60 ng/L in field samples: a) Reagent water; b) ground water; c) surface water; and d) waste water

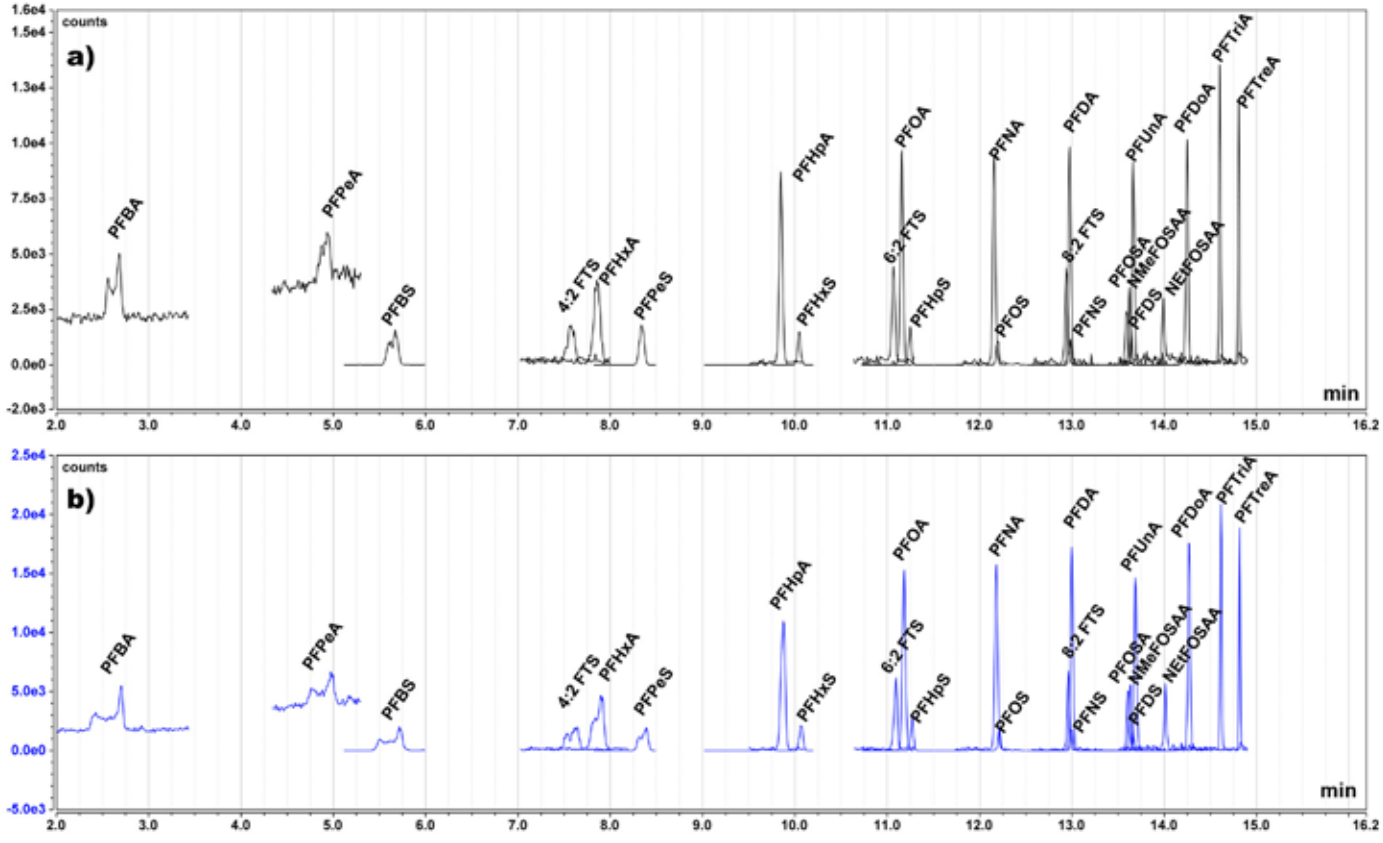

Figure 5. Overlaid chromatograms of a ground water sample spiked at 60 ng/L: a) 15 µL injection volume; b) 25 µL injection volume

Recovery of the 24 PFAS compounds spiked into the different water matrices is summarized in Table 5. All compounds analyzed in this method were within the range of 70% to 130% as required, except for PFBA spiked at low level in waste water (58% with an imprecision of 34%). The lower recovery observed may be related to co-eluting waste water matrix components causing signal suppression.

Table 5. PFAS recoveries in different water matrices, low and high levels at 60 and 200 ng/L, respectively

Compound	Recoveries %							
	Reagent water		Ground water		Surface water		Waste water	
	Low level	High level	Low level	High level	Low level	High level	Low level	High level
PFBA	77%	78%	71%	75%	74%	74%	58%	75%
PFPeA	84%	80%	104%	80%	115%	81%	88%	78%
PFBS	87%	81%	95%	81%	95%	79%	72%	77%
PFHxA	82%	81%	83%	79%	86%	80%	77%	74%
4:2 FTS	81%	82%	90%	78%	87%	79%	76%	91%
PFPeS	80%	80%	82%	79%	85%	78%	80%	83%
PFHpA	84%	81%	88%	80%	89%	80%	74%	81%
PFHxS	81%	81%	87%	78%	94%	81%	85%	85%
6:2 FTS	84%	82%	85%	80%	87%	94%	78%	79%
PFOA	83%	80%	88%	82%	123%	83%	83%	86%
PFHpS	81%	81%	84%	76%	83%	78%	79%	86%
PFNA	79%	81%	84%	80%	86%	80%	79%	82%
PFOS	91%	82%	91%	78%	93%	81%	79%	90%
8:2 FTS	85%	80%	81%	75%	76%	79%	78%	83%
PFNS	85%	75%	89%	79%	81%	76%	72%	78%
PFDA	80%	81%	86%	78%	85%	79%	74%	83%
NMeFOSAA	77%	81%	80%	77%	86%	81%	82%	84%
PFOSA	76%	76%	87%	75%	91%	75%	79%	81%
PFDS	82%	78%	89%	77%	85%	79%	72%	81%
PFUnA	76%	76%	80%	81%	75%	78%	75%	83%
NEtFOSAA	82%	79%	89%	77%	89%	81%	80%	85%
PFDoA	79%	82%	83%	78%	85%	82%	79%	85%
PFTriA	87%	86%	89%	79%	92%	91%	87%	89%
PFTreA	109%	103%	112%	91%	113%	119%	100%	110%

The LC-MS/MS method has proven to be very reproducible and robust as demonstrated by the precision values of all PFAS compounds spiked in non-drinking water matrices (N=5) summarized in Table 6.

Table 6. Reproducibility represented by % CV of 24 PFAS compounds analyzed in this method

Compound	Precision (CV, %)							
	Reagent water		Ground water		Surface water		Waste water	
	Low level	High level	Low level	High level	Low level	High level	Low level	High level
PFBA	6%	3%	23%	6%	17%	6%	34%	6%
PFPeA	9%	6%	9%	6%	25%	9%	9%	3%
PFBS	7%	4%	7%	4%	15%	3%	13%	3%
PFHxA	4%	4%	5%	3%	11%	4%	3%	10%
4:2 FTS	6%	1%	2%	4%	15%	7%	10%	18%
PFPeS	2%	4%	6%	4%	16%	3%	8%	4%
PFHpA	6%	3%	6%	5%	11%	3%	5%	3%
PFHxS	4%	5%	10%	6%	17%	4%	16%	5%
6:2 FTS	12%	4%	9%	4%	16%	14%	26%	7%
PFOA	4%	5%	8%	8%	32%	11%	12%	10%
PFHpS	12%	2%	6%	5%	14%	6%	10%	10%
PFNA	6%	4%	5%	3%	14%	3%	7%	3%
PFOS	13%	5%	5%	4%	13%	4%	5%	4%
8:2 FTS	6%	6%	11%	5%	16%	5%	8%	4%
PFNS	10%	6%	11%	4%	10%	3%	13%	5%
PFDA	4%	3%	6%	4%	19%	5%	5%	4%
NMeFOSAA	11%	7%	11%	5%	18%	4%	11%	3%
PFOSA	11%	10%	13%	5%	17%	8%	8%	5%
PFDS	10%	8%	3%	5%	13%	2%	4%	8%
PFUnA	9%	5%	3%	5%	25%	4%	8%	4%
NEtFOSAA	16%	4%	7%	5%	21%	8%	13%	5%
PFDoA	6%	5%	4%	6%	15%	8%	9%	4%
PFTriA	8%	5%	10%	6%	15%	11%	6%	5%
PFTreA	22%	14%	19%	12%	20%	23%	14%	14%

Conclusions

The method referenced in this application note shows excellent quantitative performance of the TSQ Altis mass spectrometer for PFAS direct analysis in the low ng/L range in non-drinking water matrices.

- The Accucore RP-MS column provides excellent chromatographic separation and maintains robustness in challenging water matrices.
- The TSQ Altis mass spectrometer can quantitate the majority of PFAS compounds five times lower than the LLOQ reporting requirements in ASTM D7979-17 and EPA 8327 as demonstrated by the results shown in Table 3.
- PFAS compounds were detected in the different water matrices at both low and high spike concentrations with recoveries within the range required.
- All spiked water samples, in a variety of matrices, showed RSDs below 20% for most of the PFAS compounds, demonstrating the high robustness and reproducibility of the method.

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APPLICATION NOTE

Trace analysis of pharmaceuticals and organic contaminants in water

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Guoxin Lu, Analytical Laboratory Manager, Water Quality Association, Lisle, IL

Key words

Environmental analysis, water analysis, drinking water, estrone, ibuprofen, nonylphenol, naproxen, trimethoprim, phenytoin, linuron, atenolol, PPCP, environmental monitoring, EQuan MAX Plus, TSQ Endura

Goal

To demonstrate the reliable and accurate quantitative analysis of contaminants at the pg/mL level in drinking water using the Thermo Scientific™ EQuan MAX Plus™ LC-MS system coupled to the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.

Introduction

The presence of endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) in surface water and ground water sources has been known for many years. Some of these emerging contaminants are hard to remove from the source water by current drinking water treatment techniques. Municipal water could contain trace amounts, typically part per trillion (ppt) level to part per billion (ppb) level, of certain EDCs and PPCPs. While no research results yet show that these emerging contaminants constitute a health risk at these low levels, their presence is a concern to consumers. Thus, the industry is trying to make new point-of-use drinking water treatment products that can effectively remove these contaminants from municipal water. Reliable analytical methods and instrumentation to provide qualitative and quantitative analyses of these emerging contaminants at low ppt levels are of the utmost importance. In this application note, the reporting limit (RL) of a compound is about one-sixth to one-fourth of the Maximum Effluent Concentration (MEC) of the compound in potable water.[1]

[1]Maximum Effluent Concentration (MEC) from NSF/ANSI Standard 401. The NSF/ANSI Standard sets challenge concentrations (influent concentrations) for each compound based upon the occurrence level of the contaminant in drinking water (municipal water) across the United States. In order to meet the standard, a point-of- use drinking water treatment product must remove at least 85% of the contaminant in the challenge water to meet the MEC in effluent water.

The EQuan MAX Plus LC-MS system combines a highly sensitive, online pre-concentration liquid chromatography system with the TSQ Endura triple quadrupole mass spectrometer to achieve low pg/mL level limits of quantitation with excellent quantitative reproducibility. Online pre-concentration and solid phase extraction (online SPE) avoids the disadvantages of offline SPE, including large sample volumes and preparation time, by utilizing a smaller sample volume collected in the field and eliminating the manual offline SPE step. Using this approach for analyzing for contaminants in drinking water can reduce the sample preparation time from many hours to a few minutes and still achieve ppt sensitivity.

Experimental

The EQuan MAX Plus LC-MS system was coupled to the TSQ Endura mass spectrometer.

Sample preparation

Analytical standards obtained from Restek (Catalog 569687, 569688, and 569689; Table 1) were mixed in equal proportions (Table 2) and then diluted directly into tap water from the San Jose Municipal Water System, San Jose, CA (Table 3). All dilutions to form the standard curves were made from the same San Jose tap water. No additional filtering was applied before analysis with the EQuan MAX Plus LC/MS/MS system.

Table 1. Reference standards.

Restek Standard	Compound	Stock (µg/mL)	2.5× MEC (pg/mL)	0.5× MEC (pg/mL)
569687 - Group A Standard	Ibuprofen	153.5	153.5	30.7
	Nonylphenol	534.5	534.5	106.9
	Naproxen	53.5	53.5	10.7
569688 - Estrone Standard	Estrone	53.4	53.4	10.68
569689 - Group B Standard	Atenolol	76.6	76.6	15.32
	Trimethoprim	53	53	10.6
	Phenytoin (Dilantin)	76	76	15.2
	Linuron	53	53	10.6

Table 2. Reference standards stock mixture.

Compound (-)	Stock (µg/mL)	Compound (+)	Stock (µg/mL)
Ibuprofen	51.17	Atenolol	25.53
Nonylphenol	178.17	Trimethoprim	17.67
Naproxen	17.83	Phenytoin (Dilantin)	25.33
Estrone	17.80	Linuron	17.67

Table 3. Dilutions of stock solutions in San Jose, CA, tap water.

Dilution	Stock (µg/mL)	10× MEC (pg/mL)	3.33× MEC (pg/mL)	1.11× MEC (pg/mL)	0.37× MEC (pg/mL)	0.19× MEC (pg/mL)
Compound (-)						
Ibuprofen	51.17	614.00	214.90	71.63	23.88	11.94
Nonylphenol	178.17	2138.00	748.30	249.43	83.14	41.57
Naproxen	17.83	214.00	74.90	24.97	8.32	4.16
Estrone	17.80	213.60	74.76	24.92	8.31	4.15
Compound (+)						
Atenolol	25.53	306.40	107.24	35.75	11.92	5.96
Trimethoprim	17.67	212.00	74.20	24.73	8.24	4.12
Phenytoin (Dilantin)	25.33	304.00	106.40	35.47	11.82	5.91
Linuron	17.67	212.00	74.20	24.73	8.24	4.12

Eight target compounds were selected for the analysis (Figure 1). Of these, estrone, ibuprofen, nonylphenol and naproxen are suited to negative ion LC/MS/MS analyses, and trimethoprim, phenytoin, linuron, and atenolol are suited to positive ion LC/MS/MS analyses. Samples were prepared as described in Table 4 at several concentration levels based on the target MEC.

HPLC

Water samples of 1 mL were directly injected onto a Thermo Scientific™ Hypersil GOLD™ aQ pre-concentration trapping column (2.1 × 20 mm, 12 µm, P/N 25302-022130) at 1.5 mL/min with water + 0.1% formic acid for positive ion analysis and 1.5 mL/min with water for negative ion analysis. After sufficient washing on the pre-concentration column, the target compounds were transferred at 0.4 mL/min either to a Thermo Scientific™ Accucore™ aQ analytical column (2.1 × 100 mm, 2.6 µm, positive ion analysis, P/N 17326-102130) or a Thermo Scientific™ Hypersil GOLD™ aQ analytical column (2.1 × 100 mm, 3.0 µm, negative ion analysis, P/N 25302-102130) for chromatographic separation by gradient elution prior to introduction into the mass spectrometer (Table 5).

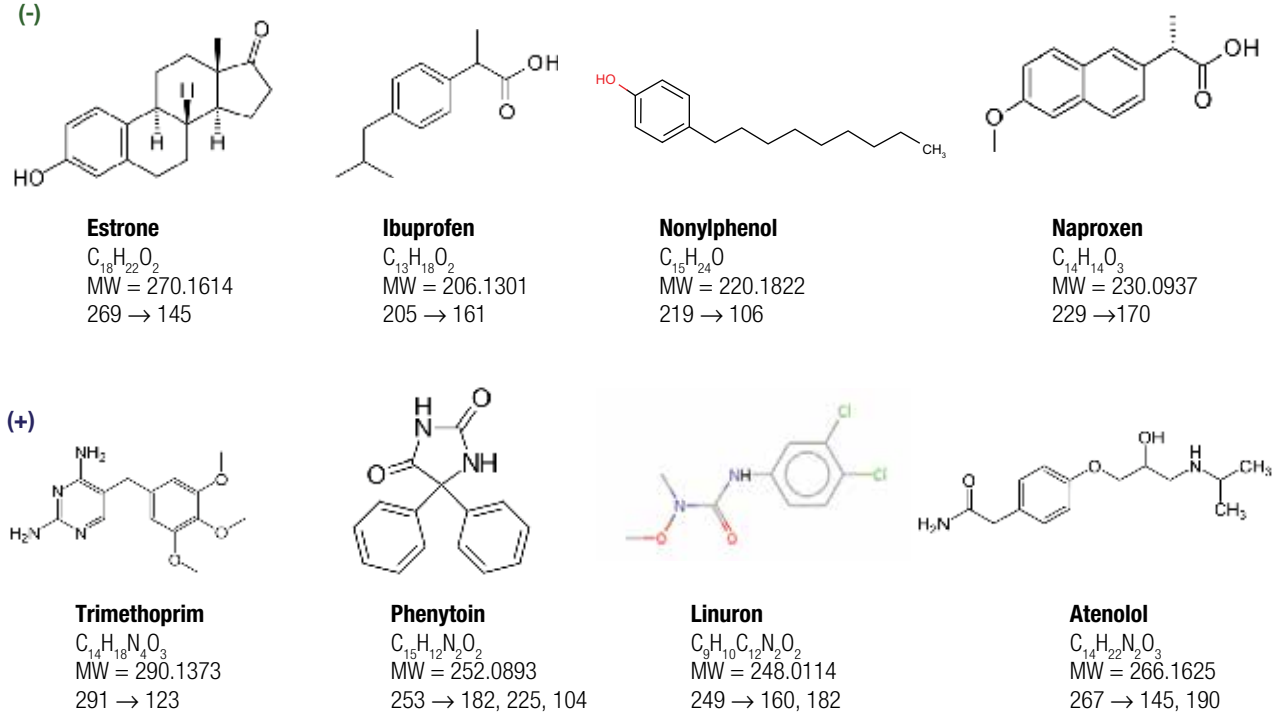

Figure 1. Target compounds.

Table 4. Sample composition.

			Sample Concentration (pg/mL)						
	Compound Name	CAS #	Sample #1 (Blank)	Sample #2 (0.5× MEC Level)	Sample #3 (2.5× MEC Level)	Sample #4 (5× MEC Level)	Sample #5 (0.5× MEC Level)	Sample #6 (2.5× MEC Level)	Sample #7 (5× MEC Level)
Group A	Estrone	53-16-7	0	11	53	106	0	0	0
	Ibuprofen	15687-27-1	0	30	152	304	0	0	0
	Nonylphenol	104-40-5	0	106	532	1064	0	0	0
	Naproxen	22204-53-1	0	11	53	106	0	0	0
Group B	Trimethoprim	738-70-5	0	0	0	0	11	53	106
	Phenytoin (Dilantin)	57-41-0	0	0	0	0	15	76	152
	Linuron	330-55-2	0	0	0	0	11	53	106
	Atenolol	29122-68-7	0	0	0	0	15	76	152

Table 5. Gradient method.

Positive Ions			
Mobile Phase	A: 0.1% formic acid in water B: 0.1% formic acid in methanol		
Gradient	*Time*	*%A*	*%B*
	0.00	100	0
	1.00	100	0
	5.00	0	100
	6.50	0	100
	6.60	100	0
	8.50	100	0
Negative Ions			
Mobile Phase	A: 0.1% ammonium hydroxide in water B: 0.1% ammonium hydroxide in methanol		
Gradient	*Time*	*%A*	*%B*
	0.00	90	10
	1.00	90	10
	3.50	0	100
	6.50	0	100
	6.60	90	10
	8.50	90	10

MS

MS analysis was carried out on a TSQ Endura triple quadrupole mass spectrometer equipped with a heated-electrospray ionization interface (H-ESI). Two selected reaction monitoring (SRM) transitions per compound were acquired: one for quantitation and the other for positive confirmation.

The MS conditions were as follows:

Parameter	*Setting*
Spray voltage	Positive: 3000 V Negative: 2000 V
Sheath gas	60
Aux gas	15
Sweep gas	1
Ion transfer tube temperature	300 °C
Vaporizer temperature	375 °C
Cycle time	0.35 s
Q1/Q3 resolution	0.7 amu
CID gas	2 mTorr
SRM transitions	Tables 6 and 7

Quantitative analysis was performed using Thermo Scientific™ TraceFinder™ software.

Table 6. SRM transitions for positive ions.

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Linuron	1	6.5	Positive	249.02	160	17	100
Linuron	1	6.5	Positive	249.02	182.02	16	100
Phenytoin	1	6.5	Positive	253.1	104.05	20	120
Phenytoin	1	6.5	Positive	253.1	182.1	15	120
Atenolol	1	6.5	Positive	267.17	145.07	25	115
Atenolol	1	6.5	Positive	267.17	190.09	19	115
Trimethoprim	1	6.5	Positive	291.15	123.07	26	125
Trimethoprim	1	6.5	Positive	291.15	230.12	24	125

Table 7. SRM transitions for negative ions.

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Ibuprofen	1	6.5	Negative	205.12	161.13	8	53
Ibuprofen	1	6.5	Negative	206.14	162.14	8	53
Nonylphenol	1	6.5	Negative	219.18	106.04	22	140
Nonylphenol	1	6.5	Negative	220.18	107.05	22	140
Naproxen	1	6.5	Negative	229.09	170.07	15	56
Naproxen	1	6.5	Negative	229.09	185.1	8	56
Estrone	1	6.5	Negative	269.15	143.05	58	225
Estrone	1	6.5	Negative	269.15	145.07	39	225

Results and discussion

Example SRM chromatograms for Group A (positive ion) and Group B (negative ion) compounds at 10× MEC are shown in Figure 2 and Figure 3, respectively. For ibuprofen and nonylphenol, a second product ion was not observed. Instead for demonstration purposes, the A+1 isotope was fragmented and its product ion was used as the confirming ion.

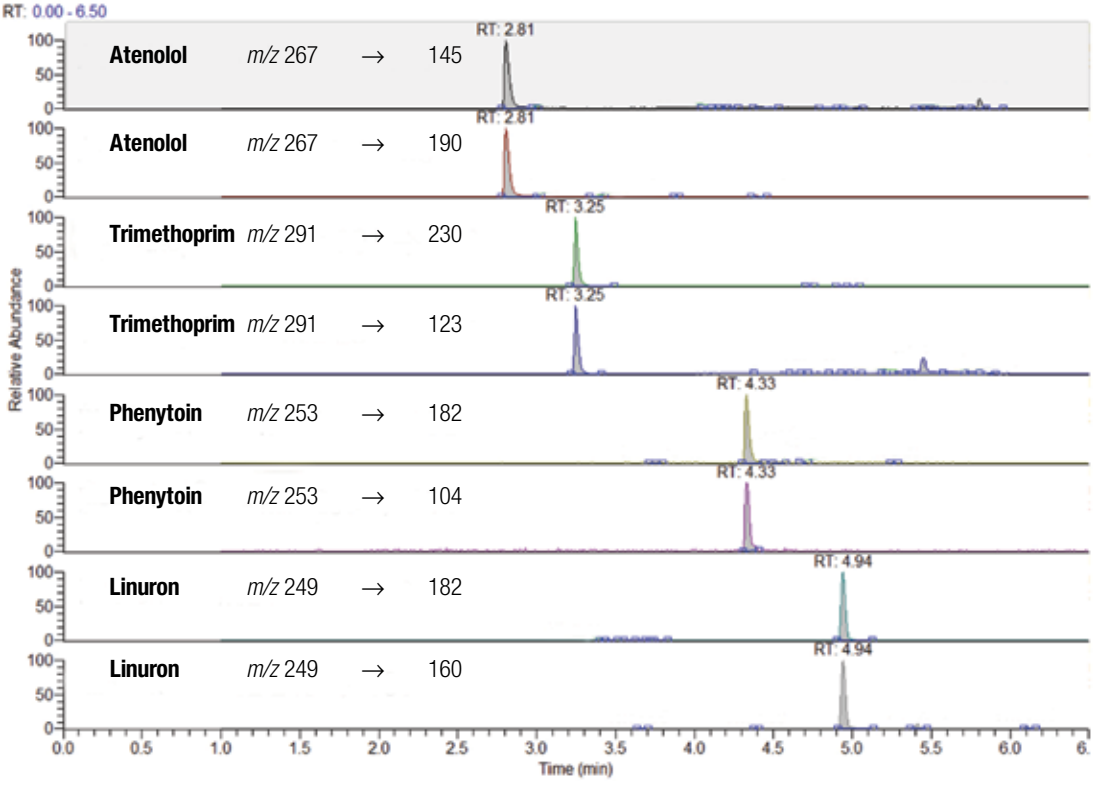

Figure 2. Positive ion SRM chromatograms (10× MEC).

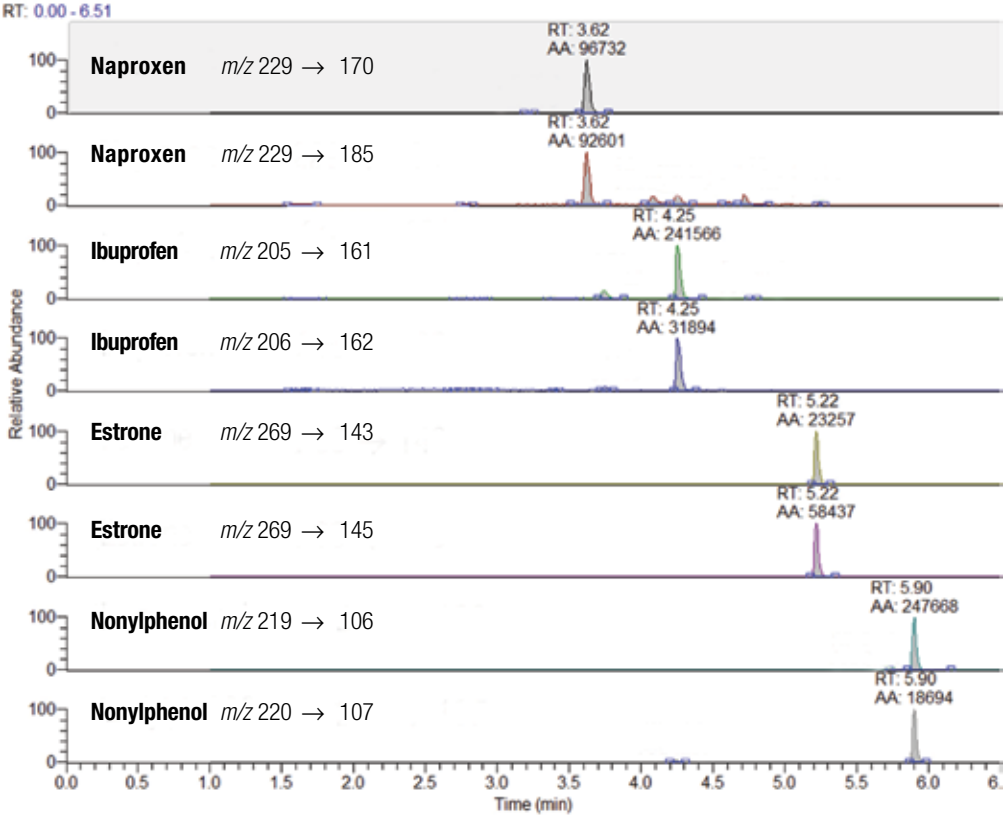

Figure 3. Negative ion SRM chromatograms (10× MEC).

Calibration curves for target organic contaminants in tap water are shown in Figures 4 and 5, which demonstrate performance to levels below MEC.

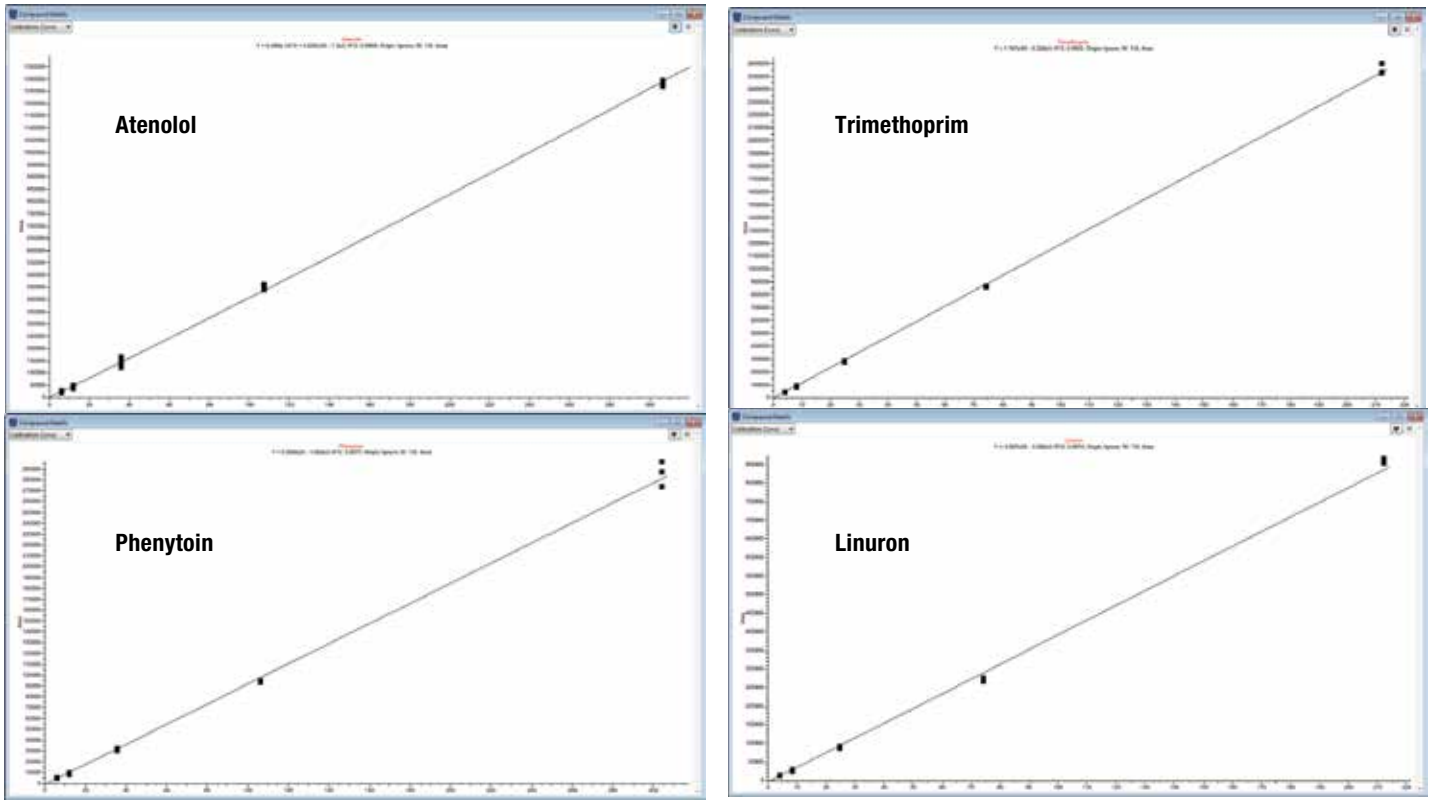

Figure 4. Standard calibration curves for postive ion compounds.

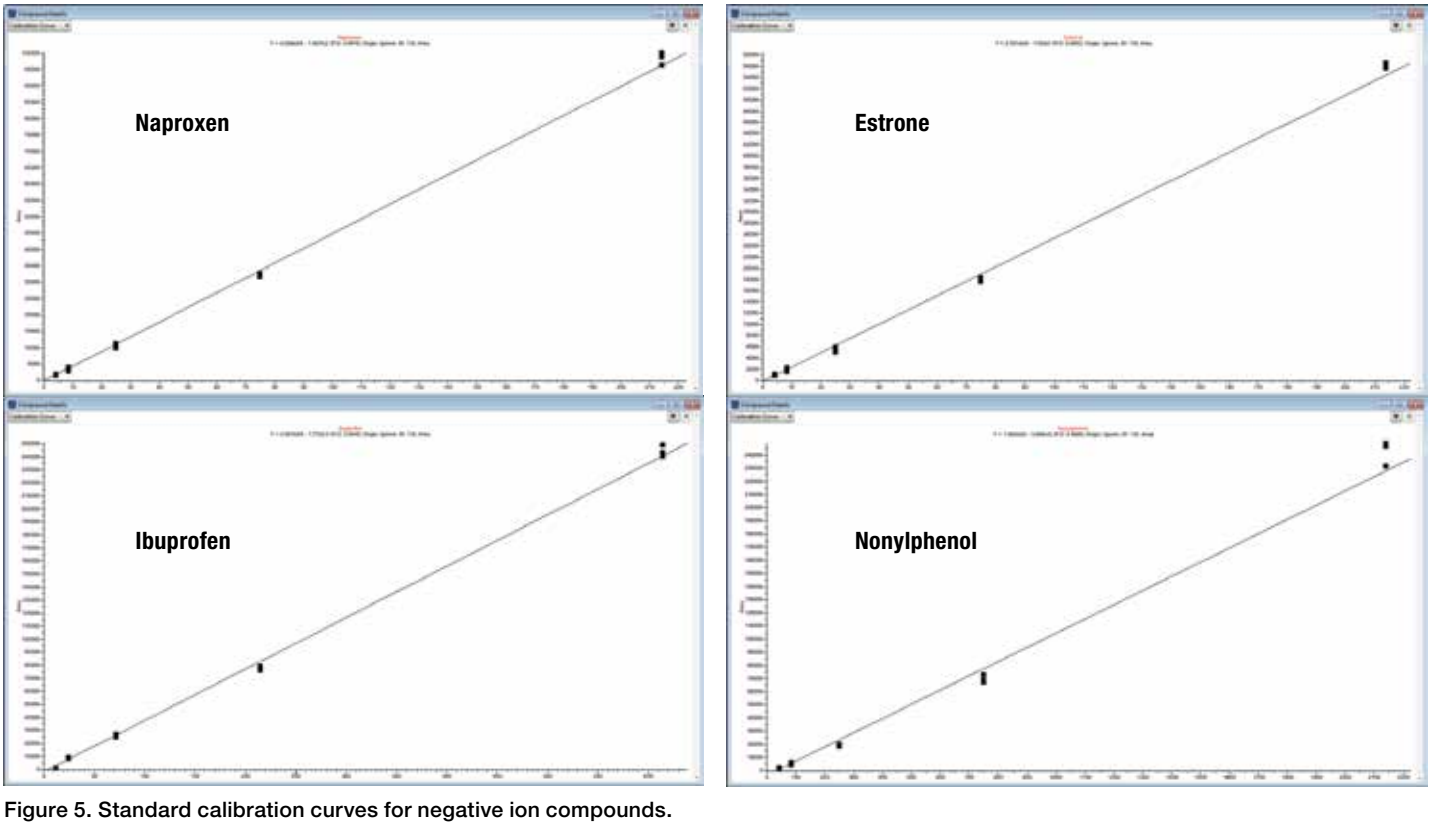

Figure 5. Standard calibration curves for negative ion compounds.

Tables 8 and 9 show system reproducibility as %RSDs for N=7 replicate injections for spiked tap water and customer-submitted samples, respectively. The EQuan MAX Plus LC-MS system demonstrated excellent reproducibility for the target compounds in water at 0.37× MEC in spiked tap water using 1 mL injections. Several compounds showed a significantly lower response in the customer water samples versus the spiked tap water at a similar concentration (for example, >90% loss for trimethoprim and ibuprofen, and nonylphenol yielding erratic results barely distinguisable from the matrix blanks). This may be due to sample degradation and/or sample adsorption losses. It is clear that these samples need to be analyzed fresh, and not stored.

Table 8. Reproducibility for freshly prepared San Jose tap water samples.

Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
0.37× MEC	38822	28437	10100	44582	0.37× MEC	3574	1736	8707	5340
0.37× MEC	44001	28137	8129	47123	0.37× MEC	3864	1865	9115	6011
0.37× MEC	46701	27867	10171	47820	0.37× MEC	3636	2089	9140	5321
0.37× MEC	49224	28763	9764	49229	0.37× MEC	4099	1817	9654	5650
0.37× MEC	45401	25168	9220	47522	0.37× MEC	3600	2316	9134	6155
0.37× MEC	47108	26598	9577	50476	0.37× MEC	3795	2341	9423	5306
0.37× MEC	41807	29374	9499	48323	0.37× MEC	3595	2061	8863	5854
Average	**44723.4**	**27763.4**	**9494.3**	**47867.9**	**Average**	**3737.6**	**2032.1**	**9148.0**	**5662.4**
RSD	**7.86%**	**5.16%**	**7.25%**	**3.85%**	**RSD**	**5.20%**	**11.75%**	**3.48%**	**6.24%**
Conc	**Atenolol**	**Linuron**	**Phenytoin**	**Trimethoprim**	**Conc**	**Naproxen**	**Estrone**	**Ibuprofen**	**Nonylphenol**
1.11× MEC	135637	92213	30762	148796	1.11× MEC	10435	5884	25247	20106
1.11× MEC	165827	90151	31562	148943	1.11× MEC	11296	5902	25967	19574
1.11× MEC	134388	88078	30732	146316	1.11× MEC	10764	5447	25933	20118
1.11× MEC	128029	88202	30999	143493	1.11× MEC	11206	5579	27224	20216
1.11× MEC	149999	87276	31073	147697	1.11× MEC	10431	5835	26132	19773
1.11× MEC	139983	87589	30450	147524	1.11× MEC	10374	6015	26701	19520
1.11× MEC	124696	88837	32708	149571	1.11× MEC	10069	5079	27037	20207
Average	**139794.1**	**88906.6**	**31183.7**	**147477.1**	**Average**	**10653.6**	**5677.3**	**26320.1**	**19930.6**
RSD	**10.09%**	**1.95%**	**2.43%**	**1.40%**	**RSD**	**4.28%**	**5.81%**	**2.66%**	**1.51%**

Table 9. Reproducibility for customer-supplied water samples.

Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
0.5× MEC	34248	20349	8696	1303	0.5× MEC	1647	236	148	6416
0.5× MEC	32075	21716	8235	1546	0.5× MEC	1331	236	192	4494
0.5× MEC	31781	17293	7549	1223	0.5× MEC	1730	256	325	4133
0.5× MEC	28063	19953	8268	1338	0.5× MEC	1351	227	236	3273
0.5× MEC	21982	19352	8452	1645	0.5× MEC	1371	258	295	3575
0.5× MEC	22017	19292	7535	1805	0.5× MEC	1474	327	2395	3095
0.5× MEC	34238	20793	7810	2377	0.5× MEC	942	175	1421	3045
Average	29200.6	19821.1	8077.9	1605.3	Average	1406.6	245.0	716.0	4004.4
RSD	18.27%	7.05%	5.60%	24.78%	RSD	18.19%	18.56%	120.60%	29.79%
Conc	Atenolol	Linuron	Phenytoin	Trimethoprim	Conc	Naproxen	Estrone	Ibuprofen	Nonylphenol
2.5× MEC	174508	113980	40225	12788	2.5× MEC	7032	776	2056	8434
2.5× MEC	178278	114151	42507	13232	2.5× MEC	4873	677	2012	9585
2.5× MEC	182584	115500	43456	13444	2.5× MEC	7510	835	2440	1147
2.5× MEC	188987	115823	43815	13195	2.5× MEC	6072	710	1726	7226
2.5× MEC	182180	118057	44245	13136	2.5× MEC	5321	631	1146	7150
2.5× MEC	187777	113413	44513	10081	2.5× MEC	4748	761	1190	7958
2.5× MEC	183085	114405	38815	10571	2.5× MEC	5302	701	1939	8690
Average	182485.6	115047.0	42510.9	12349.6	Average	5836.9	727.3	1787.0	7170.0
RSD	2.76%	1.37%	5.13%	11.36%	RSD	18.44%	9.38%	26.48%	38.89%

Conclusion

- The TSQ Endura triple quadrupole mass spectrometer in concert with the EQuan MAX Plus online pre-concentration liquid chromatography system proves to be a reliable and accurate system for the quantitative analysis of contaminants at the pg/mL level in drinking water.
- Samples prepared freshly from reference standard stock solutions show better performance than those prepared and stored for significant periods.
- Excellent reproducibility was shown for the target compounds in tap water using 1 mL injections at 0.37× MEC.
- Using timed-SRM, where target compounds are measured only during a specific time window, the reproducibility (%RSDs) near the LOD would be improved.

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