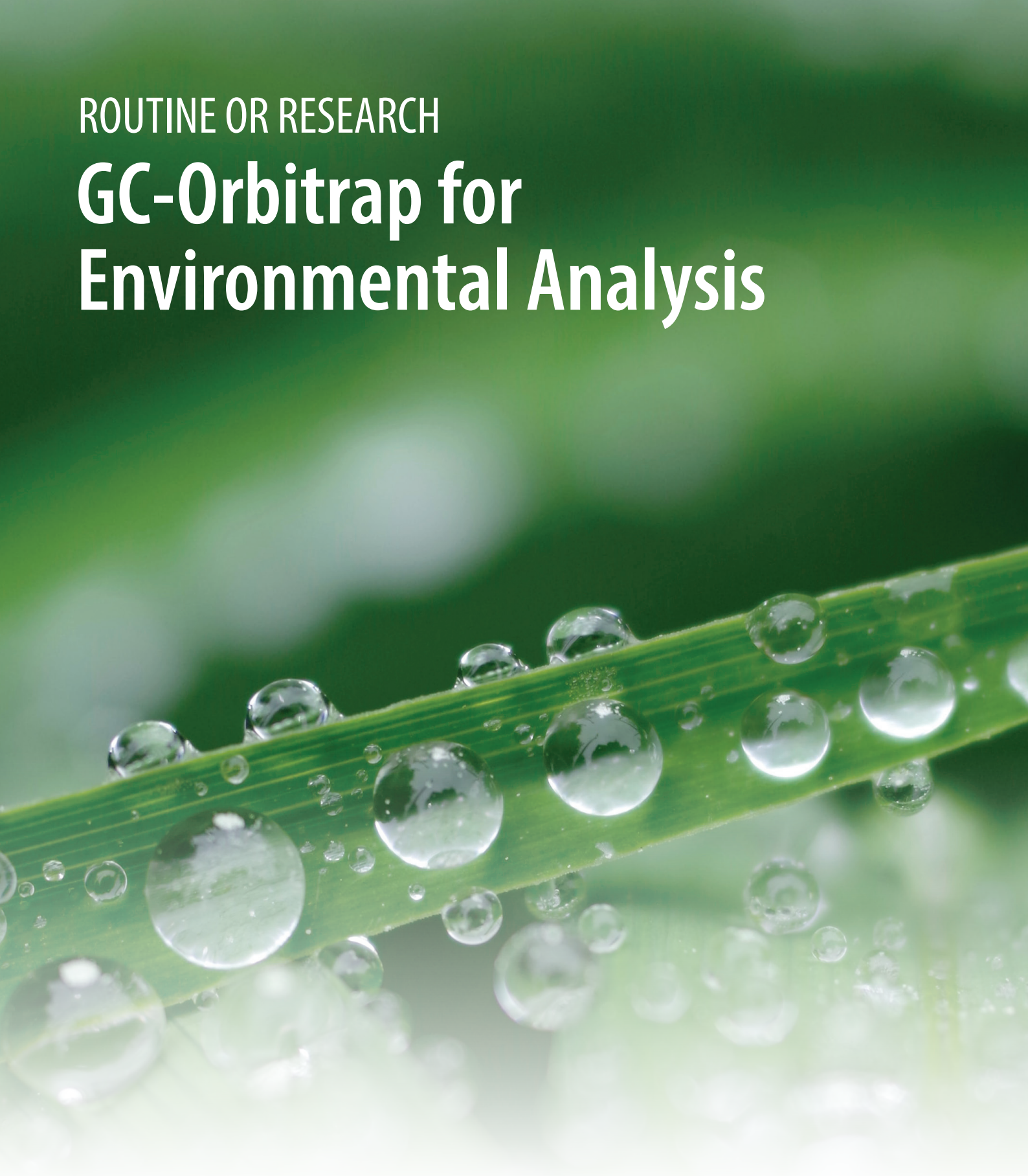
ROUTINE OR RESEARCH

GC-Orbitrap for Environmental Analysis



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GC-Orbitrap for Environmental Analysis

Foreword

A Novel High Resolution Accurate Mass Orbitrap-based GC-MS Platform for Routine Analysis of Short Chained Chlorinated Paraffins

In this study, the performance of a novel bench top, high resolution accurate mass Orbitrap™-based GC-MS was tested for the analysis of SCCPs. System performance was tested using full-scan acquisition and simple instrumental setup.

Pyrolysis-GC-Orbitrap MS - A Powerful Analytical Tool for Identification and Quantification of Microplastics in a Biological Matrix

The purpose of the experiments described in this work was to assess the applicability of pyrolysis-gas chromatography-Orbitrap™ mass spectrometry for the qualitative and quantitative analysis of plastic polymers in complex biological matrices.

Low Level Quantification of NDMA and Non-targeted Contaminants Screening in Drinking Water using GC Orbitrap Mass Spectrometry

In this work, a sensitive and selective method for NDMA detection and quantification using high resolution accurate mass GC Orbitrap™ technology is described.

Overcoming Analytical Challenges for Polybrominated Diphenyl Ethers (PBDEs) Analysis in Environmental Samples using Gas Chromatography – Orbitrap Mass Spectrometry

The note demonstrates 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.

Versatility of GC-Orbitrap Mass Spectrometry for the Ultra-trace Detection of Persistent Organic Pollutants in Penguin Blood from Antarctica

In this study, the performance of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ mass spectrometer was evaluated for routine analysis of POPs within King penguin blood from Antarctica.

Discovery of Emerging Disinfection By-Products in Water Using Gas Chromatography Coupled with Orbitrap-based Mass Spectrometry

In this work, a novel gas chromatography, coupled with high-resolution accurate mass Orbitrap™ mass spectrometer has been used for iodo-DBPs detection and accurate mass identification in chlorinated and chloraminated water samples.

Full-scan Analytical Performance Exactive GC and the Q Exactive GC Mass Spectrometers

The objective of this study was to test the analytical performance of the Exactive™ GC system and the Q Exactive™ GC system using full-scan acquisition. Both mass spectrometers were evaluated for key analytical parameters such as scan speed, sensitivity, mass accuracy, dynamic range and linearity.

Case Study: GC Orbitrap MS/MS System Addresses an Expanding Set of Compounds of Concern to Health and the Environment

In this case study Dr Flavio Ciesa (Agenzia Provinciale per l'ambiente) explains how the Q Exactive™ GC Orbitrap™ GC-MS/MS system brings together the power of GC and HRAM Orbitrap™ MS to provide high-capacity targeted and untargeted component detection, even in extremely complex samples.

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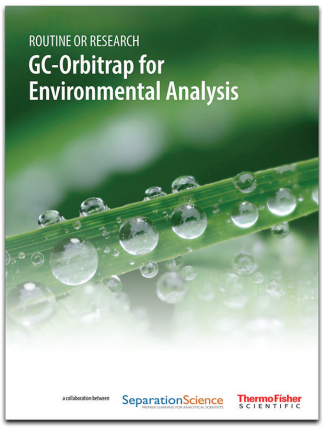
GC-Orbitrap for Environmental Analysis

Foreword



A wide variety of contaminants with the potential to cause harm to humans and animals can make their way into the environment. They can be found in the air, water and soil and may come from sources such as industrial waste, landfill sites, pesticides and pharmaceutical drugs. Identifying these contaminants is challenging because of the huge variety of potential compounds with varying chemical compositions. These challenges call for sophisticated analytical techniques, such as Orbitrap-based high-resolution mass spectrometry, which provide distinct advantages.

Since the launch of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS in 2015 and the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS System one year later, environmental scientists and analysts have had the ability to obtain an unprecedented depth in the analysis of their samples. Whether their goals have been targeted routine analysis of contaminants or research into emerging compounds, the power of high-resolution full-scan analysis has opened up new possibilities.

For routine analysis GC-Orbitrap offers distinct benefits including consolidation of multiple methods onto a single system, increasing the scope of analysis to keep pace with changing regulations and the ability to retrospectively detect compounds. For research applications, the power to confidently and accurately identify unknown compounds is critical to the advancement of knowledge into contaminant levels and their environmental pathways. Once detected, accurate and precise quantification is delivered with ease through the wide dynamic range provided by Orbitrap technology.

In this new eBook we bring together some of the key application notes, written in collaboration with scientists from around the world, which demonstrate how GC-Orbitrap makes a real difference to environmental analysis.

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TECHNICAL NOTE 10539

A novel high resolution accurate mass Orbitrap-based GC-MS platform for routine analysis of Short Chained Chlorinated Paraffins

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Keywords

Exactive GC, Persistent Organic Pollutants (POPs), Short Chained Chlorinated Paraffins (SCCPs), environment, emerging contaminants, sensitivity, mass accuracy, high resolution, Orbitrap technology, gas chromatography

Introduction

Short Chained Chlorinated Paraffins (SCCPs) are emerging contaminants that, once released, will remain in the environment for long periods of time with the potential to bioaccumulate in living organisms. SCCPs are intentionally manufactured and used as lubricants and coolants in the metal processing industry or as plasticizers and flame retardants in plastic products. Chronic exposure to SCCPs is believed to have harmful and irreversible effects for humans and the environment.[1] As a consequence, SCCPs are listed in the Stockholm convention as chemicals with potential adverse effects and their production and use in Europe is restricted and regulated.[2]

Detection and quantification of SCCPs poses analytical challenges due to the fact that these compounds are present in the environment at low levels, as very complex isomeric mixtures and are difficult to separate chromatographically. Although there is no consensus for the use of a validated analytical procedure for the routine monitoring of SCCPs in environmental samples, there are several analytical methods that are used to detect and quantify SCCPs. Details of these methods and their limitations are listed in Table 1.

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Table 1. Current analytical methodology used for the analysis of SCCPs.

Carbon skeleton analysis by GC-FID or GC-MS	GC-ECD	GC-NCI-MS
Details: Uses Pd catalyst held in the gas chromatograph injector to simultaneously dechlorinate the CPs and separate the resulting alkanes	**Details:** GC coupled to electron capture detector, sensitive for halogenated compounds	**Details:** Uses soft ionisation (negative chemical ionisation) with methane and/or dichloromethane
Disadvantages: • Lack in sensitivity and selectivity • No information on the degree of chlorination of the SCCPs can be achieved	**Disadvantages:** • Relatively non-specific • Interferences from other halogenated compounds	**Disadvantages:** • Low resolution GC-MS nominal mass interferences from higher chlorinated PCBs, toxaphenes and chlordane-related compounds, have similar molecular masses

In this study, the performance of a novel bench top, high resolution accurate mass Orbitrap-based GC-MS was tested for the analysis of SCCPs. System performance was tested using full-scan acquisition and simple instrumental setup. The experiments performed focused on assessing the sensitivity, linear dynamic range, selectivity and analytical precision for the analysis of two SCCPs technical mixtures. Both electron ionization (EI) and negative chemical ionization (NCI) were used and the results compared and discussed.

Experimental

In the experiments described here, a Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph for gas-phase separation of target compounds, achieved on a Thermo Scientific™ TraceGOLD TG5-SilMS 15m x 0.25 mm x 0.25 µm column (P/N 26096-1300). Injection of liquid samples was performed automatically using a Thermo Scientific™ TriPlus™ RSH™ autosampler. The Exactive GC was tuned and calibrated in under one minute using PFTBA to achieve the best ion transmission and sub-ppm mass accuracy. The mass spectrometer was operated in full-scan using 60k mass resolution (measured as FWHM at *m/z* 200). Lockmass corrected data was processed with Thermo Scientific™ TraceFinder™ software. Additional details regarding the GC and MS conditions are given in Table 2.

Table 2. Gas chromatography and mass spectrometers analytical parameters.

TRACE 1310 GC System Parameters	
Injection Volume (µL):	2.0
Liner:	LinerGOLD™, single taper (P/N:453A0344-UI)
Inlet (°C):	280
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	100
Hold Time (min):	2.0
Temperature 2 (°C):	310
Rate (°C/min):	20
Hold Time (min):	4.0

Electron Ionization MS Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full-scan
Mass range (Da):	50-650
C-Trap voltage (V):	0.0
Mass resolution (FWHM at *m/z* 200):	15k, 30k and 60k
Lockmass (*m/z*):	207.03235 281.05114 355.06993

Negative Chemical Ionization MS Parameters	
Transfer line (°C):	280
Ionization type:	NCI
Ion source (°C):	200
Reagent gas and flow (mL/min):	Methane, 1.2
Electron energy (eV):	70
Electron Lens Voltage (V):	10
Emission current (µA):	150
C-Trap voltage:	2.0
Acquisition Mode:	Full-scan
Mass range (Da):	200-550
Mass resolution (FWHM at *m/z* 200):	60k
Lockmass (*m/z*):	234.94104

Complete separation of SCCPs is very difficult due to the very high numbers of isomers and homologues with similar physiochemical properties (Figure 1). Due to the fragmentation obtained in EI, it is often difficult to find homologue specific ions with sufficient intensity for use as quantification masses, highly efficient electron ionization can be used to detect and quantify SCCPs.

To provide even higher selectivity for homologue groups, GC-MS with negative chemical ionization is the method preferred by many laboratories for the detection and quantification of SCCPs. NCI allows for sensitive and selective detection of SCCPs by using ions characteristic for various homologue groups in a mixture (in particular [M-Cl]-, [M-HCl]-). Using NCI, the SCCP congeners in the two technical mixtures were easily separated based on the number of chlorine substitutes for a certain carbon chain length and according to the number of carbon atoms and chlorine atoms for various carbon chain lengths. Examples of congener specific extracted ion chromatograms are given in Figure 2.

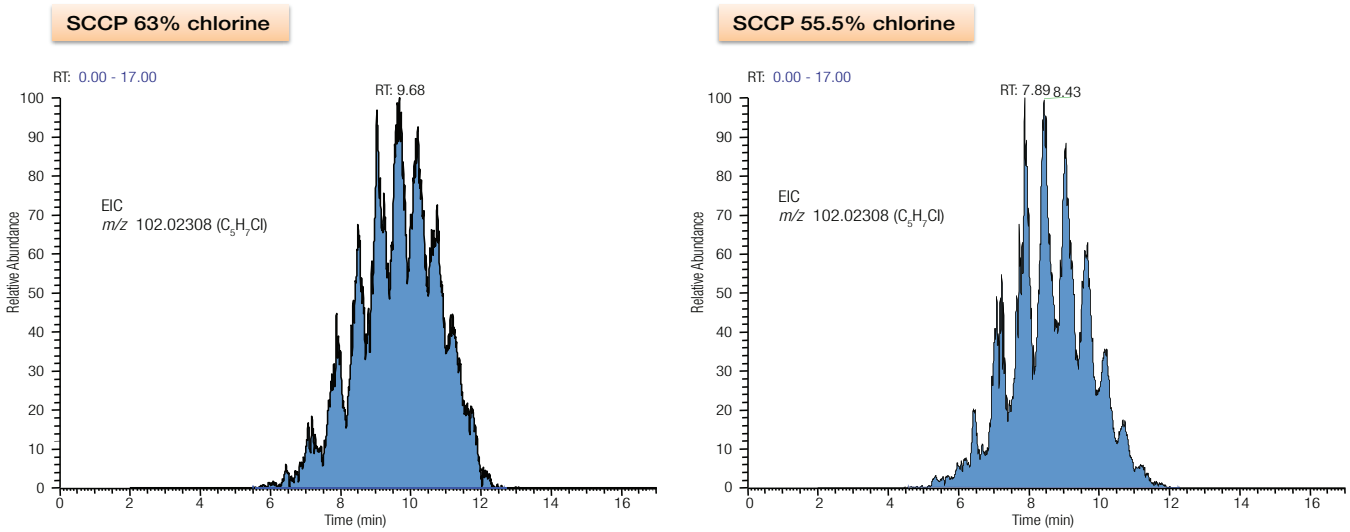

Figure 1. Extracted ion chromatogram of the fragment ion *m/z* 102.02308 corresponding to $C_5H_7Cl_7$, ±5ppm extraction window) showing the chromatographic complexity of two SCCP technical mixtures (63% and 55.5% chlorine). Data acquired in EI, full-scan, using 60k resolution.

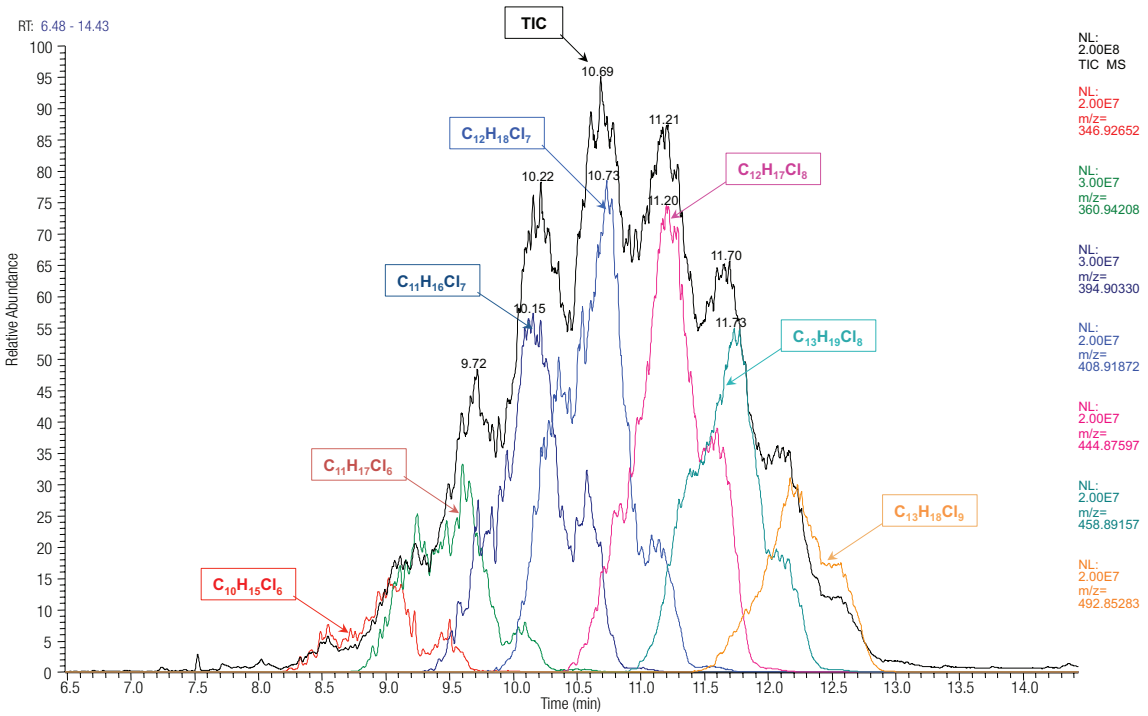

Figure 2. High resolution accurate mass selectivity demonstrated for the C_{10}-C_{13} 63% technical mix acquired in NCI at 60k resolution. Examples of extracted ion chromatograms for individual homologues with various chlorination degrees are shown.

Sensitivity

The sensitivity of the Exactive GC was tested by injecting low concentration solvent standards (in cyclohexane) prepared by a serial dilution of the two SCCP technical mixtures (Figure 3). The limit of detection varies depending on the relative concentration of a particular congener in a technical mixture. From the EI data, the instrumental detection limits (IDL) were ~10 pg/µL (calculated as total homologues response for each of the two SCCPs technical mixtures). In addition, the NCI data demonstrates that IDLs as low as 3 pg/µL can be obtained for individual homologue groups (Table 3).

Table 3. Peak area repeatability calculated as %RSD from n=10 repeat injections at 25 pg/µL level for two SCCP congeners acquired using NCI. IDL calculated taking into account the Student's-t critical values for the corresponding degrees of freedom (99% confidence).

inj. no	*m/z* 492.8546 ($C_{13}H_{18}Cl_9$)	*m/z* 458.8936 ($C_{13}H_{19}Cl_8$)
1	765881	1308232
2	822551	1428540
3	795041	1361253
4	781911	1363928
5	776597	1321808
6	731874	1250508
7	761201	1305483
8	749797	1284342
9	737987	1257718
10	757772	1286412
mean	768061	1316822
StDev	27217	54540
%RSD	**3.5**	**4.1**
IDL	**2.5**	**2.9**

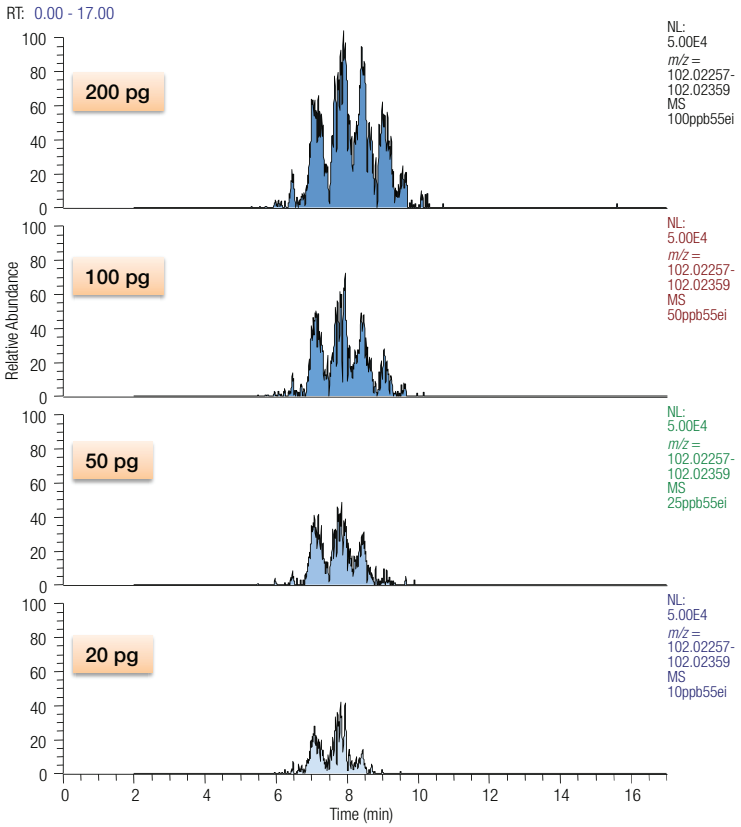

Figure 3. Extracted ion chromatograms (*m/z* 102.02308, mass window ±5ppm) representing the total SCCP C_{10}-C_{13} 55.5% chlorine homologues. Peak area response at 20, 50, 100 and 200 pg on column concentrations) are shown.

Linearity and dynamic range

SCCPs linearity and dynamic range was assessed for each SCCP technical mix (63% and 55.5%) using the following dilution series: 1, 10, 25, 50, 100, 250, 500, 1000, 5000 and 10000 pg/µL (in cyclohexane). This test was performed using both EI and NCI and examples are given in Figure 4. The coefficient of determination was >0.99 indicating excellent linearity across this concentration range.

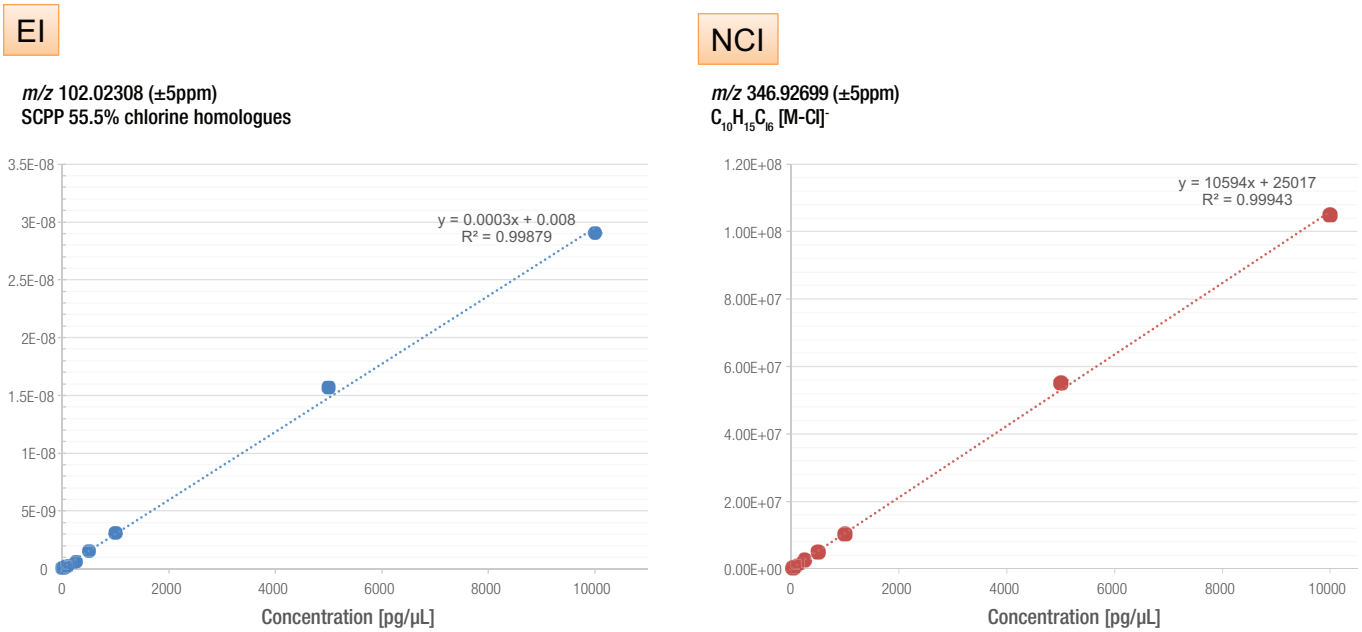

Figure 4. Example of linearity obtained for the C_{10}-C_{13} 55.5% chlorine homologues across 10 – 10000 pg/µL in EI and NCI at 60k resolution.

Moreover, selected individual homologue masses showed excellent linearity when acquired in NCI with R^2 values >0.999 (Figure 5).

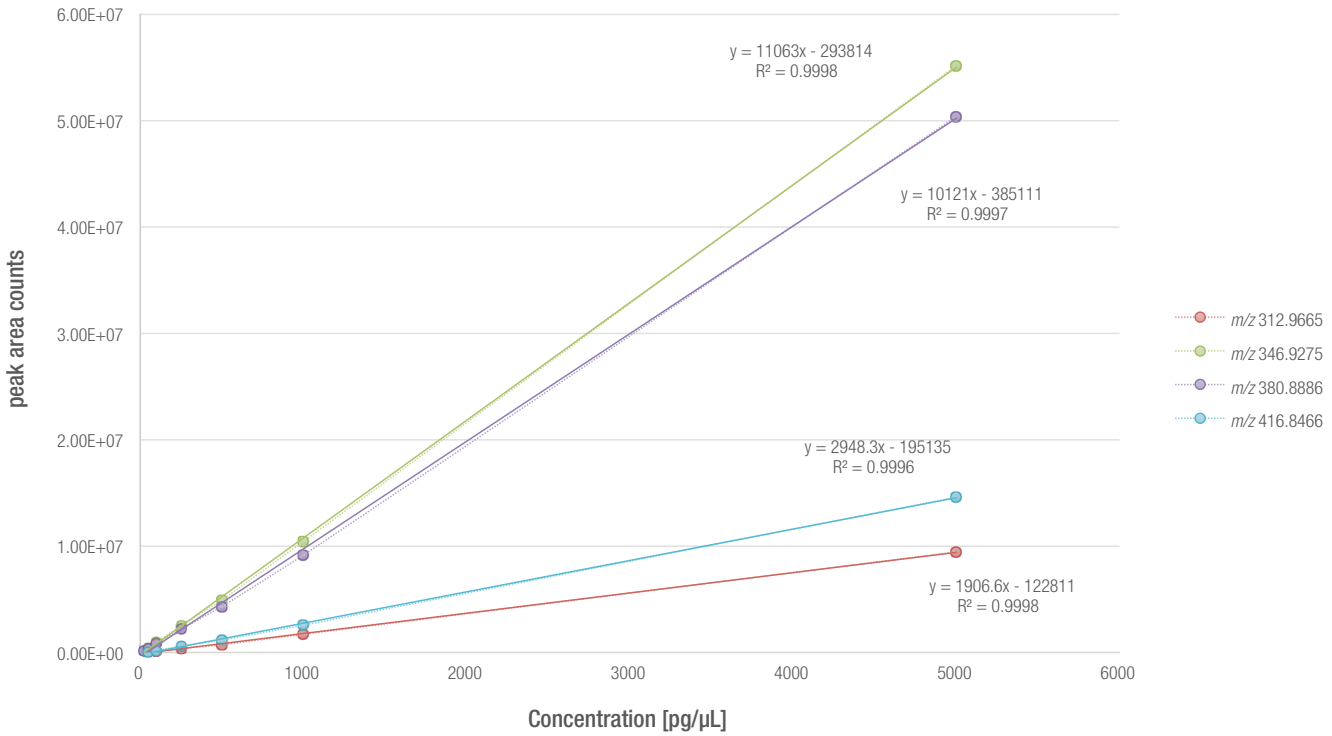

Figure 5. Example of linearity obtained individual SCCP congeners in the C_{10}-C_{13} 63% technical mixture across 1 – 5000 pg/µL concentration range. Data acquired in full-scan, NCI at 60k resolution.

Selectivity through high mass resolution

High resolution and high mass accuracy enable excellent selectivity and specificity. This is particularly important for full-scan data when background masses such as matrix ions, other organochloro contaminants or ions from other SCCP homologous group interfere with the target masses, leading to erroneous quantification.

As demonstrated in Figure 6 for a SCCPs 55% chlorine standard spiked with polychlorinated biphenyls, a resolving power of 15k is not sufficient to differentiate between an SCCP ion (*m/z* 253.03121, $C_{11}H_{16}Cl_3$) and a PCB interference (*m/z* 253.01733, $C_{14}H_{11}Cl_2$). Instead, at 15k resolution a single ion is detected, which in turn will significantly affect the peak area determination and precise estimation of SCCP concentration. At low resolving power, the extracted peak area of the target SCCP ion *m/z* 253.03121 is significantly lower than those obtained at 30k or 60k resolution due to higher errors in mass measurements (ppm). To achieve the sub-ppm mass accuracy and the selectivity required for consistent separation and quantification of target compounds, resolving powers of >30k are needed.

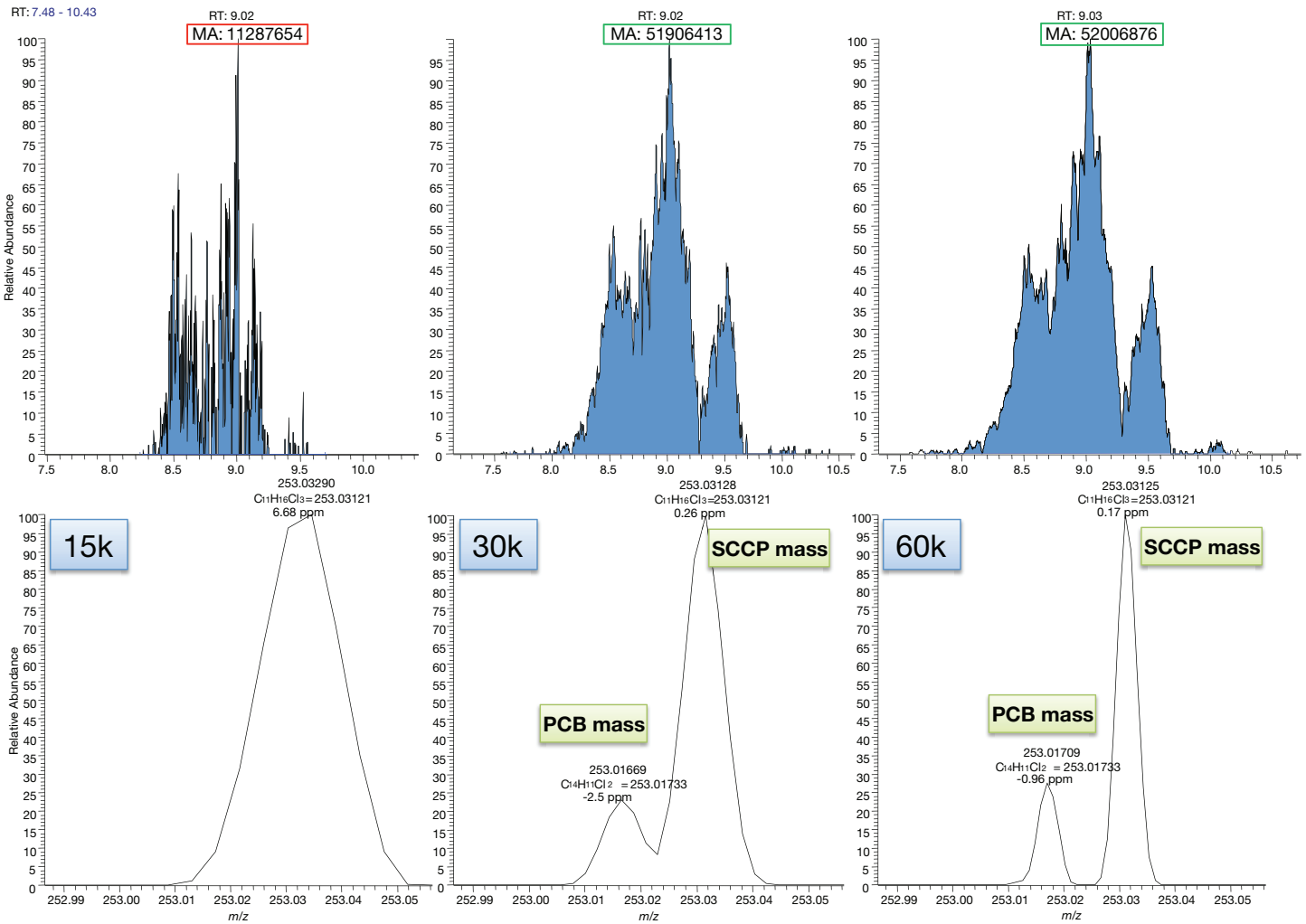

Figure 6. Selectivity enhancement by using narrow mass tolerance windows is possible at high resolving power. The effect of increasing resolving powers of the mass accuracy and peak area of a SCCP ion *m/z* 250.03121 is demonstrated for a 55% SCCPs sample spiked with polychlorinated biphenyls. Data acquired in full-scan, EI.

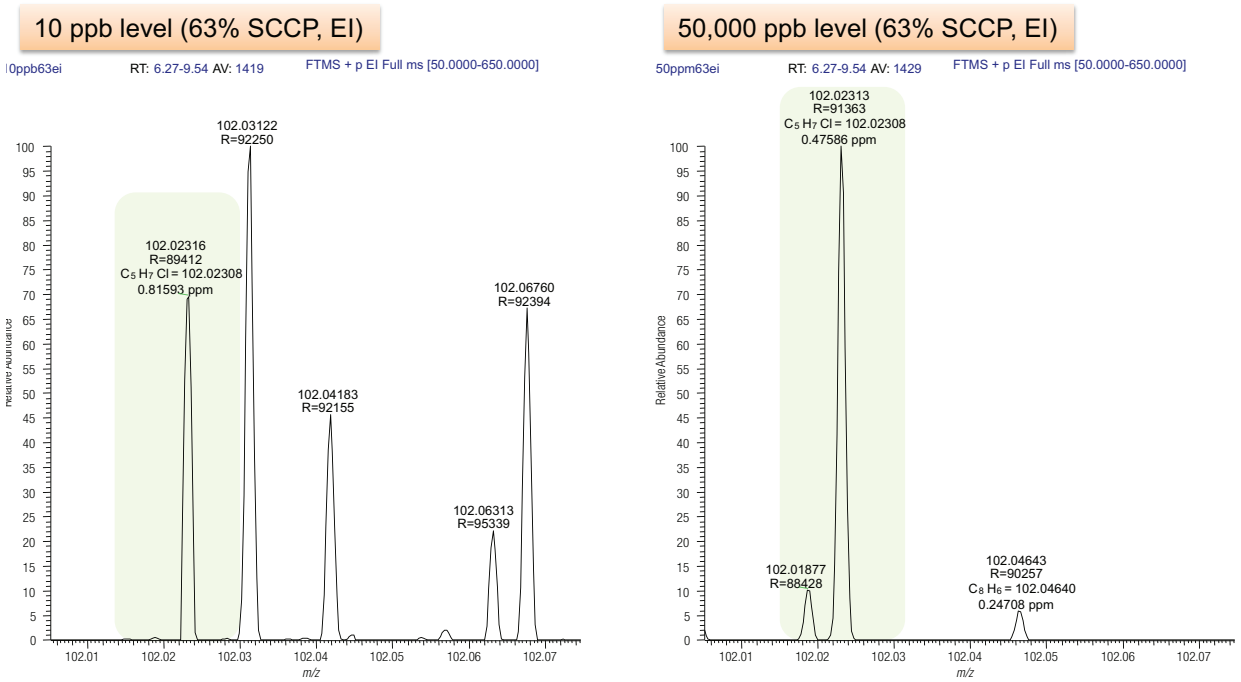

Figure 7. Mass accuracy remained at <1 ppm level irrespective of the by compound concentration as demonstrated for SCCPs fragment ion *m/z* 102.02308 (C_5H_7Cl) acquired in full-scan, EI at 60,000 mass resolution. The exact mass resolution, as well as the mass precision (ppm), used are annotated to each measured ion.

Maintaining sub-ppm mass accuracy at low and high concentrations

Outstanding mass accuracy (<1 ppm) was maintained across all compound concentrations (Figure 7). This is essential, as any compromise in accuracy of mass measurements can result in false identification, erroneous quantification and interferences from matrix ions.

Conclusions

These preliminary results demonstrate that the Exactive GC is a potential solution to address the difficult challenges related to the detection and quantification of SCCPs due to the excellent sensitivity, linearity and selectivity and in combination with an uncomplicated instrumental setup.

From the EI data, low instrumental LODs of ~10 pg/µL, calculated as total homologues response for each of the two SCCPs technical mixtures, can be easily obtained.

Using NCI it is possible to selectively separate C10 alkanes chains with various chlorination degrees making quantification of homologues with similar Cl content achievable.

In addition, with increased selectivity, the NCI data demonstrates that LOQs as low as 3 pg/µL can be reliably obtained for individual homologue groups.

Excellent linearity was obtained across a total SCCP mixture concentration range of 1 – 10,000 pg/µL, making the Exactive GC an ideal quantification tool.

The high resolving power of the Exactive GC facilitates sub-ppm mass accuracy at low and high concentrations, essential for achieving enough selectivity to confidently separate SCCP specific low mass ions from the interfering background ions (in EI), or higher masses (in NCI), for various SCCP homologue groups.

References

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APPLICATION NOTE 10643

Pyrolysis-GC-Orbitrap MS - a powerful analytical tool for identification and quantification of microplastics in a biological matrix

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Keywords

Exactive GC, pyrolysis, microplastics, high resolution, Orbitrap technology, gas chromatography, fishmeal

Goal

The purpose of the experiments described in this work was to assess the applicability of pyrolysis-gas chromatography-Orbitrap™ mass spectrometry for the qualitative and quantitative analysis of plastic polymers in complex biological matrices.

Introduction

Plastics are synthetic organic polymers, commercially introduced on a large scale starting in the 1950s. Single-use plastics (grocery bags, food packaging, bottles, utensils) are persistent pollutants making up approximately 40% of beach litter[1]. This litter eventually ends up in the marine environment, with an estimated 8 million metric tons of plastic waste entering the oceans worldwide every year[2]. Most plastics have a very long degradation time, and for a timespan up to centuries they end up as macro-, micro- and nanoplastics through weathering. Due to their characteristics and additional content (monomeric residue, plasticizers, flame retardants etc.), micro- and nanoplastics can have complex toxicological effects on marine life through direct ingestion[3-5] and/or leachates[6]. This might represent a hazard for ecosystems and for human exposure through consumption and inhalation[7]. As this is an emerging field, there are limited studies on the identification

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of plastic polymers, and stringent quantification requirements remain to be developed. Estimates of plastic loads in the oceans range six orders of magnitude, while no comprehensive data exist for microplastics in soils despite considerable agricultural use[8].

Among the analytical techniques used for the analysis of microplastics are Fourier Transform Infrared (FTIR), Raman spectroscopy and microscopy and also pyrolysis-gas chromatography mass spectrometry (py-GC-MS). Py-GC-MS presents a promising approach for surveillance where throughput is critical. Furthermore, this analytical approach would enable time-saving detection of bulk amounts of micro- and nanoplastics below the lower size limit of the microscopy techniques. Therefore, low detection limit, dynamic range, and linearity as well as high compound selectivity and measurement uncertainty are crucial.

In this study, the efficiency of pyrolysis GC coupled to high-resolution, accurate-mass spectrometry was investigated for the qualitative and quantitative analysis of microplastics, as opposed to single quadrupole, which has been used in previously published work[9]. The sample pyrolyzer was connected to a bench top, high-resolution accurate-mass Orbitrap-based GC-MS system that facilitates the detection and quantification of low level compounds against a complex chemical background. The experiments described here focused on preliminary assessment of the power of accurate mass for the characterization of plastic polymers as well as the quantitative performance of this analytical configuration.

Experimental

Sample preparation

Custom-made known plastics standards were obtained through participation in the BASEMAN research project, which is financed as part of Joint Programming Initiative (JPI) Oceans, through the Norwegian Research Council (NFR). Aliquots of polymethyl methacrylate (PMMA) and polystyrene (PS) standards, dissolved in ethyl acetate, were transferred to pyrolyzer cups with the final weight of each polymer in each cup approximately 0.05, 0.5, 5, and 50 µg. To investigate whether these polymers could be detected in a more complex matrix easily, fishmeal was decomposed using 10% KOH (w/w) at 50 °C followed by 30% H_2O_2 (w/w) at 40 °C. Between 0.3 and 0.5 g fishmeal resulted in about 5–10 mg decomposed material, which was spiked with 2.5 µg PMMA and 2.7 µg PS. To evaluate the qualitative properties of the py-GC-MS, samples of solid polymers of polyamides (PA), polycarbonate (PC), polyethylene (PE), PMMA, polypropylene (PP), PS, polyvinyl chloride (PVC), and poly(ethylene terephthalate) (PET) and mixtures thereof (10–100 µg of each polymer) were weighed into pyrolyzer cups. To all samples, 10 µL tetramethylammonium hydroxide (TMAH; Sigma-Aldrich; 25%, v/v) was added as a methylating agent before analysis.

Instrumental analysis

The Frontier Lab's Multi-Shot Pyrolyzer™ (Frontier EGA/PY-3030D) with Auto-Shot Sampler™ (AS-1020E) was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N 26096-1420). The GC system was then coupled to a Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer (Figure 1). The Exactive GC system was tuned and calibrated in under one minute using PFTBA to achieve the best ion transmission and sub-ppm mass accuracy. The mass spectrometer was operated in full-scan mode using 60,000 mass resolution (measured as FWHM at *m/z* 200). Lockmass corrected data was processed with Thermo Scientific™ TraceFinder™ software. Additional details regarding the pyrolysis, GC, and MS conditions are given in Tables 1 and 2.

Figure 1. Instrumental setup: Multi-Shot Pyrolyzer (Frontier EGA/PY-3030D) with Auto-Shot Sampler (AS-1020E) coupled to an Exactive GC Orbitrap mass spectrometer

Table 1. GC and injector conditions

TRACE 1310 GC System Parameters	
Injector:	Thermo Scientific™ Instant Connect Thermospray (TSI)
Inlet:	270 °C
Carrier Gas:	He, 1.2 (mL/min)
Split Flow:	200 mL/min
Oven Temperature Program	
Temperature 1:	50 °C
Hold Time:	1 min
Temperature 2:	320 °C
Rate:	15 °C/min
Hold Time:	5 min
Multi-Shot Pyrolyzer EGA/PY-3030D Parameters	
Oven Temp.:	600 °C
Interface Temp. :	300 °C

Table 2. Mass spectrometer conditions

Exactive GC Orbitrap Mass Spectrometer Parameters	
Transfer Line:	320 °C
Ionization Type:	EI
Ion Source:	280 °C
Electron Energy:	70 eV
Emission Current:	20 µA
Acquisition Mode:	Full-scan, centroid
Mass Range:	50-650 Da
Resolving Power:	60,000 FWHM at *m/z* 200
Lockmass, Column Bleed:	207.03235 *m/z*

Data processing

Data was acquired in full-scan centroid mode using TraceFinder software, version 4.1. This is a single software platform that allows instrument control, method development functionality, and qualitative and quantitation-focused workflows. TraceFinder software also contains spectral deconvolution and spectral matching functionality.

Results and discussion

The applicability of the Exactive GC Orbitrap GC-MS system in combination with pyrolysis for qualitative and quantitative assessment of microplastics was tested using both standards and fishmeal that were spiked with known amounts of plastic polymers. Indicator exact masses, characteristic for the plastic compounds, were extracted using different *m/z* windows to demonstrate advantages of high-resolution, accurate-mass capabilities for this application.

Sensitivity, selectivity and linearity

Linearity was tested using PS and PMMA standards with the concentration points of 0.05, 0.5, 5, and 50 µg, which is a range that corresponds to amounts found in real samples analyzed with pyrolysis-GC-MS (Kögel et al. unpublished). Excellent linear responses were obtained for both compounds with coefficient of determinations R^2 >0.999 and %RSD for residuals <15% (Figure 2-1). Using the GC-MS conditions described in Tables 1 and 2, the number of scans/chromatographic peak exceed 25 scans for a 3.5-second-wide peak for PS, and 20 scans for PMMA, allowing for and enabling accurate peak integration and compound quantification (Figure 2-2).

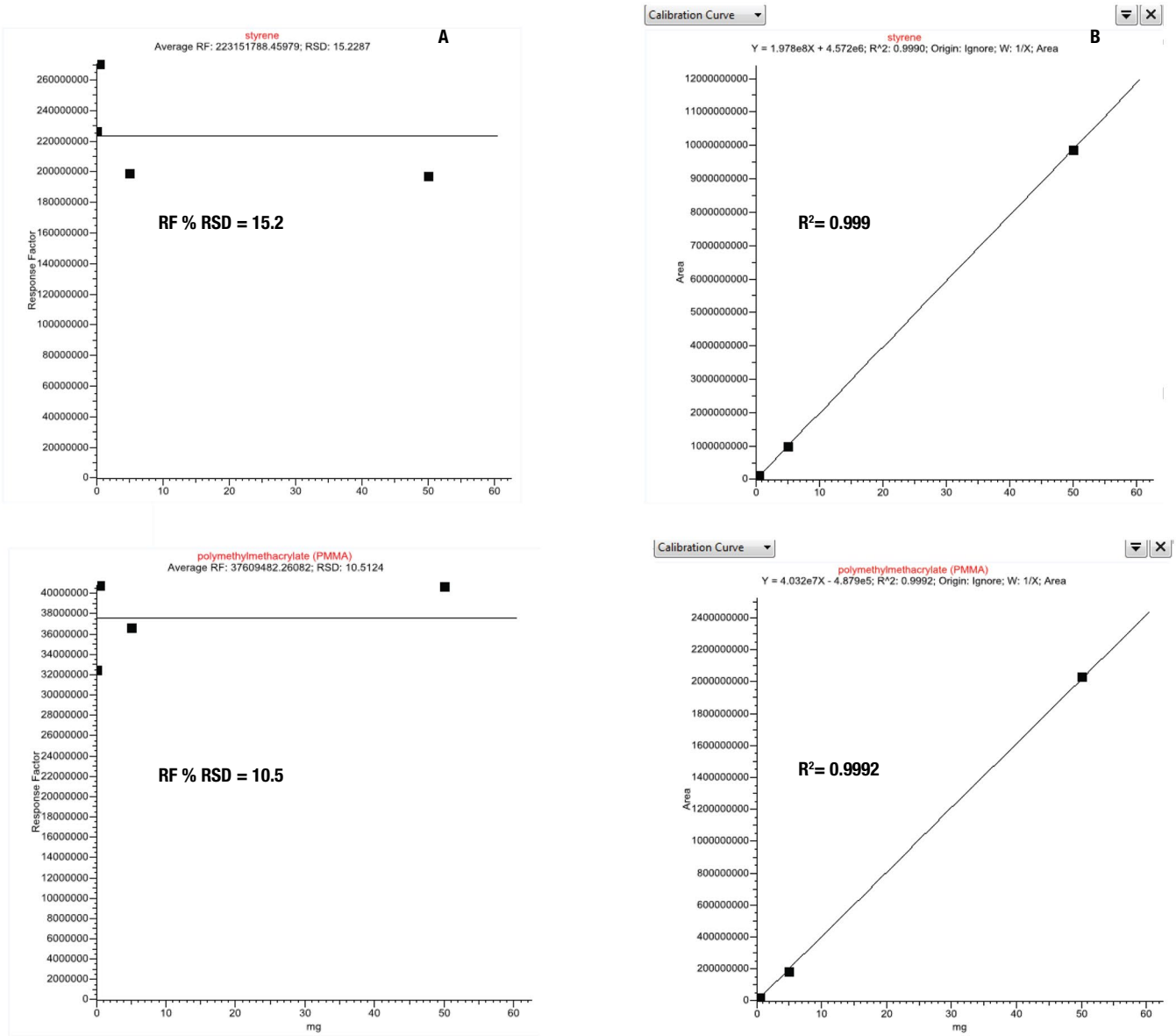

Figure 2-1. Chromatography and linearity of styrene (top) and methyl methacrylate (bottom) in a 0.05 µg standard. Extracted ion chromatograms of styrene (*m/z* 104.0621) and methyl methacrylate (*m/z* 99.0441) were used to assess the linearity of response (R^2 and RF %RSD residuals) over four concentration points 0.05–50 µg (A and B).

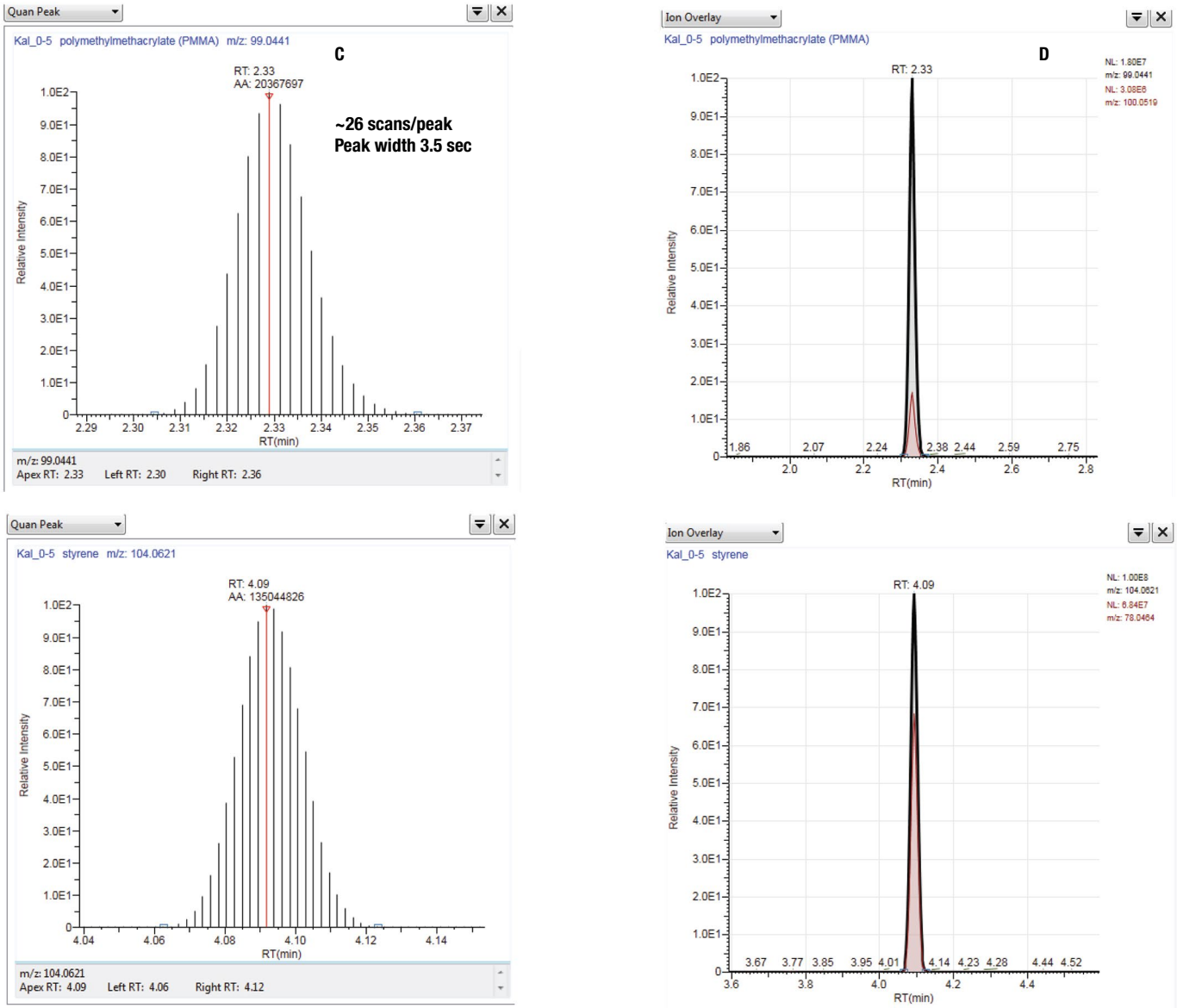

Figure 2-2. Chromatography and linearity of methyl methacrylate (top) and styrene (bottom) in a 0.05 µg standard. C shows integrated peak area of the quantification ion with corresponding scans/peak, and D shows an overlay of the quantification ion and the confirmation ion. Data were acquired in full-scan at 60,000 resolution (FWHM at *m/z* 200). Peak retention time (RT) as well as peak area counts (AA) are annotated. Peak smoothing (5× moving average was applied).

Consistent mass accuracy

In addition to the quantification performance, the mass accuracy of target compounds was assessed across all the concentrations. Obtaining accurate mass information is critical to avoid misidentification and erroneous quantification. For all compounds targeted, the mass accuracy was < 1 ppm irrespective of matrix complexity or concentration level. Figure 3 shows an example of consistently high mass accuracy maintained for all ions in PS spectra measured in the lowest (0.05 µg) and at the highest standard (50 µg).

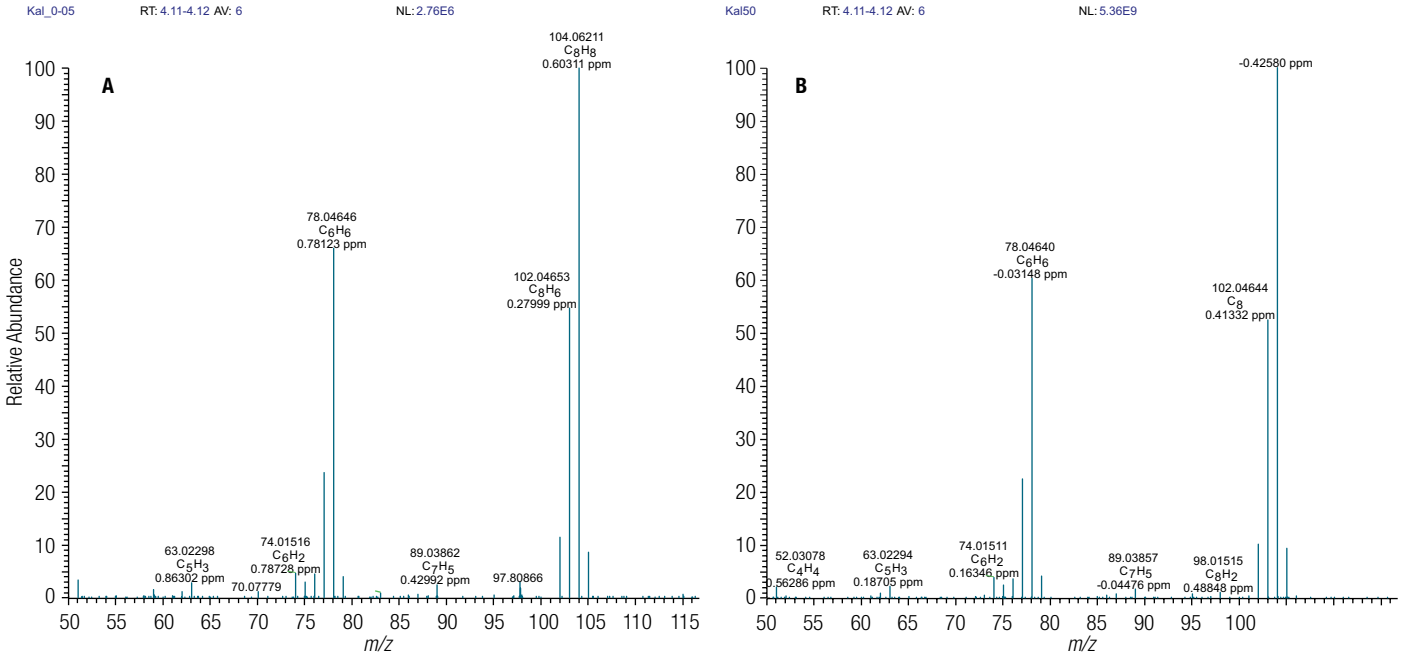

Figure 3. Stability of spectral fidelity and mass accuracy irrespective of compound concentration demonstrated for PS (styrene monomer) at 0.05 µg (A) and at 50 µg level (B)

Quantification of microplastics in a decomposed fishmeal sample spiked with PS and PMMA

To assess the accuracy of quantification of microplastics using the Exactive GC Orbitrap py-GC-MS approach, a fishmeal sample was spiked with known amounts of PS and PMMA (Table 3) and back calculation of these amounts was performed using individual PS and PMMA external standard calibration curves over the range of 0.05 to 50 µg. Even without using internal standard correction, the average % deviation from the expected results was good (-2.3% as average deviation calculated for PS and PMMA) for analysis in complex matrixes (Table 3).

Table 3. Accuracy of quantification demonstrated for PS and PMMA spiked into a decomposed fishmeal sample. Quantification of PS was done using the sum of *m/z* 104.06211 + *m/z* 91.05418, whereas PMMA was quantified using the sum of *m/z* 99.04407 + *m/z* 100.05188.

Compound	Spiked Amount (µg)	Measured Amount (µg)	% Deviation
Polystyrene (PS)	2.7	2.9	+7.4
Polymethyl methacrylate (PMMA)	2.5	2.2	-12.0

Selectivity

By using the Exactive GC Orbitrap py-GC-MS operated at routine 60,000 resolution, it is possible to selectively isolate *m/z* values corresponding to pyrolysis products of various polymers. Examples of selectivity for PA, PC, PE, PMMA, PP, PS, PVC, and PET are shown in Figures 4 and 5. Typical fragment masses used to identify and quantify the different plastic materials differ from *m/z* 78 (PVC) to *m/z* 228 (PC).[9] The minimum mass difference that is meaningful to use when extracting the quantifier ions is a function of the resolution used.

Figure 4 shows extracted quantifier ions; the ions are extracted at ±5 ppm, just above the smallest mass difference that can be resolved with a resolution of 60,000. The many characteristic peaks of the x-meric pyrolytic products of the polymer types with very small monomers (PP and PE) are clearly visible. Another important feature is the clearly peaking multimers of PS, which are necessary for selective quantification of PS, excluding the monomeric styrene, which is a pyrolysis product also deriving from natural marine chitin[9].

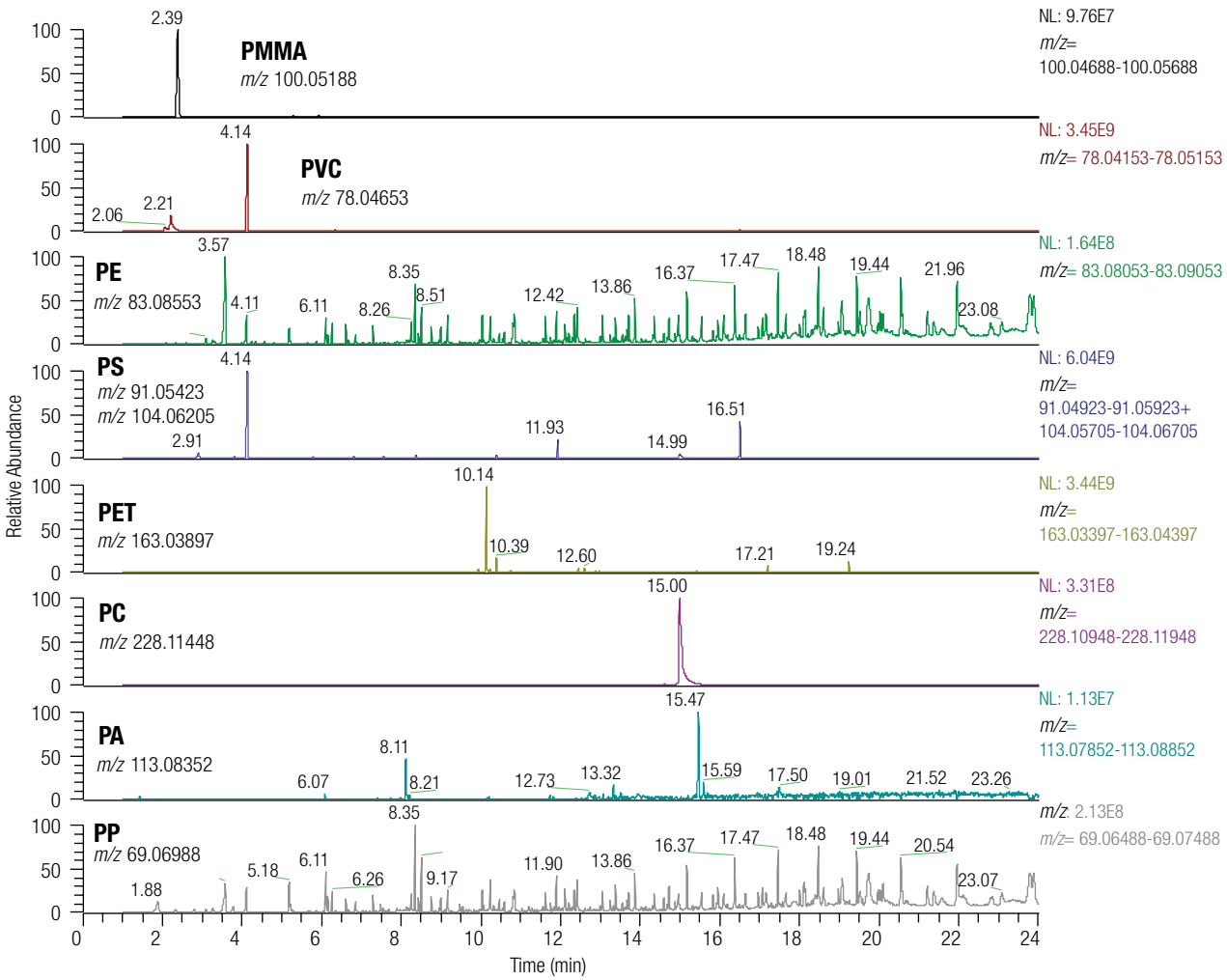

Figure 4. Selectivity for PMMA, PVC, PE, PS, PET, PC, PA, and PP polymers demonstrated as extracted ion chromatograms of each polymer using a ±5 ppm extraction window

In addition, the importance of high-resolution accurate-mass mass spectrometry for selectivity is demonstrated in Figure 5 for PA and Figure 6 for PS. Using an extraction window of >±100 mmu to simulate a low-resolution mass spectrometer, crowded extracted ion chromatograms are seen. When taking advantage of the accurate mass measured by using an extraction window of ±2 ppm, the chromatographic peaks selected for quantification are more evident.

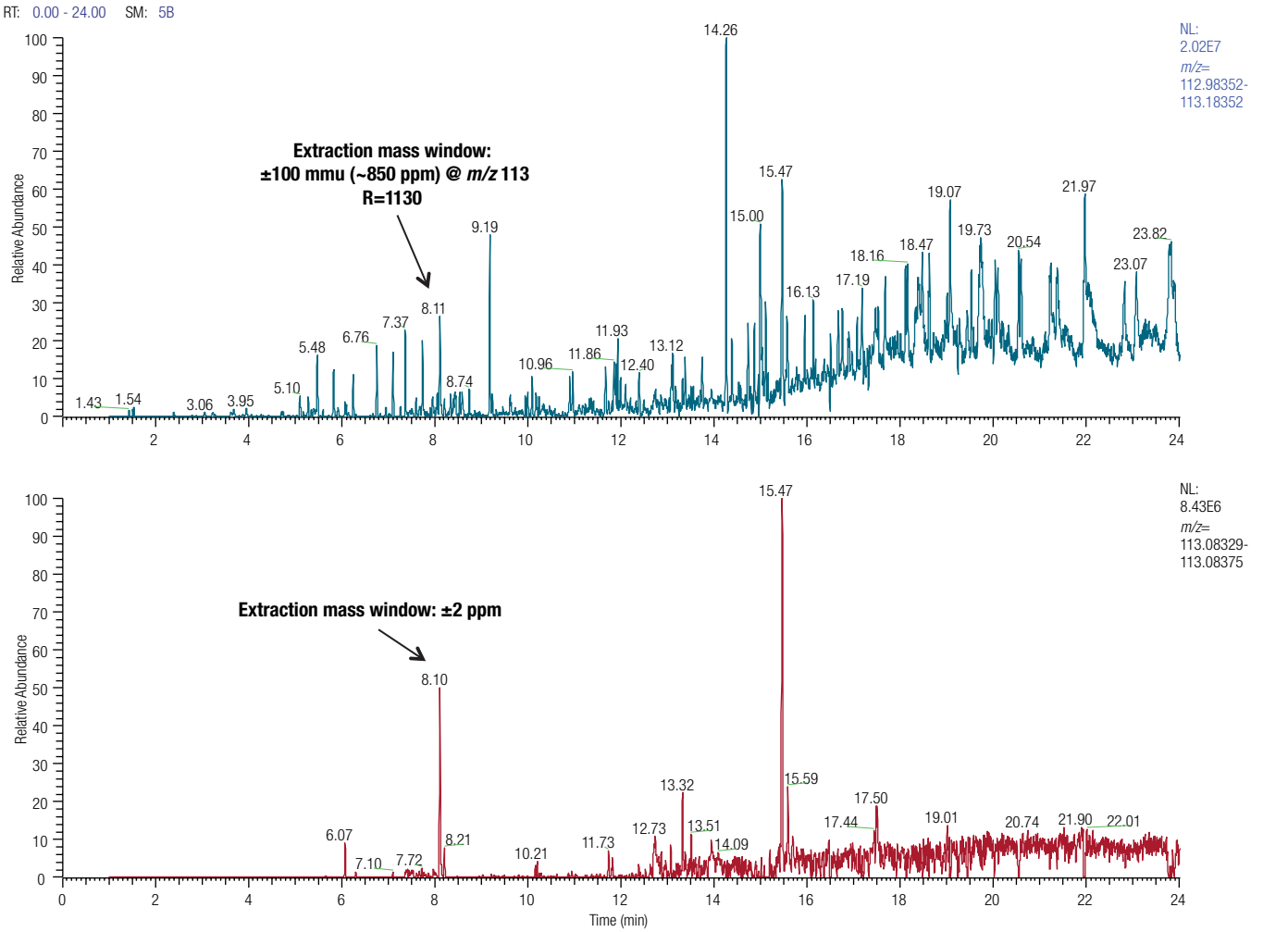

Figure 5. Selectivity for PA fragments, RT = 8.10 min, using an extraction window for *m/z* 113.08352 of ±100 mmu (equivalent to ~850 ppm) simulating a mass resolution of ~1100 (top) and ±2 ppm taking advantage of the accurate mass measured (bottom)

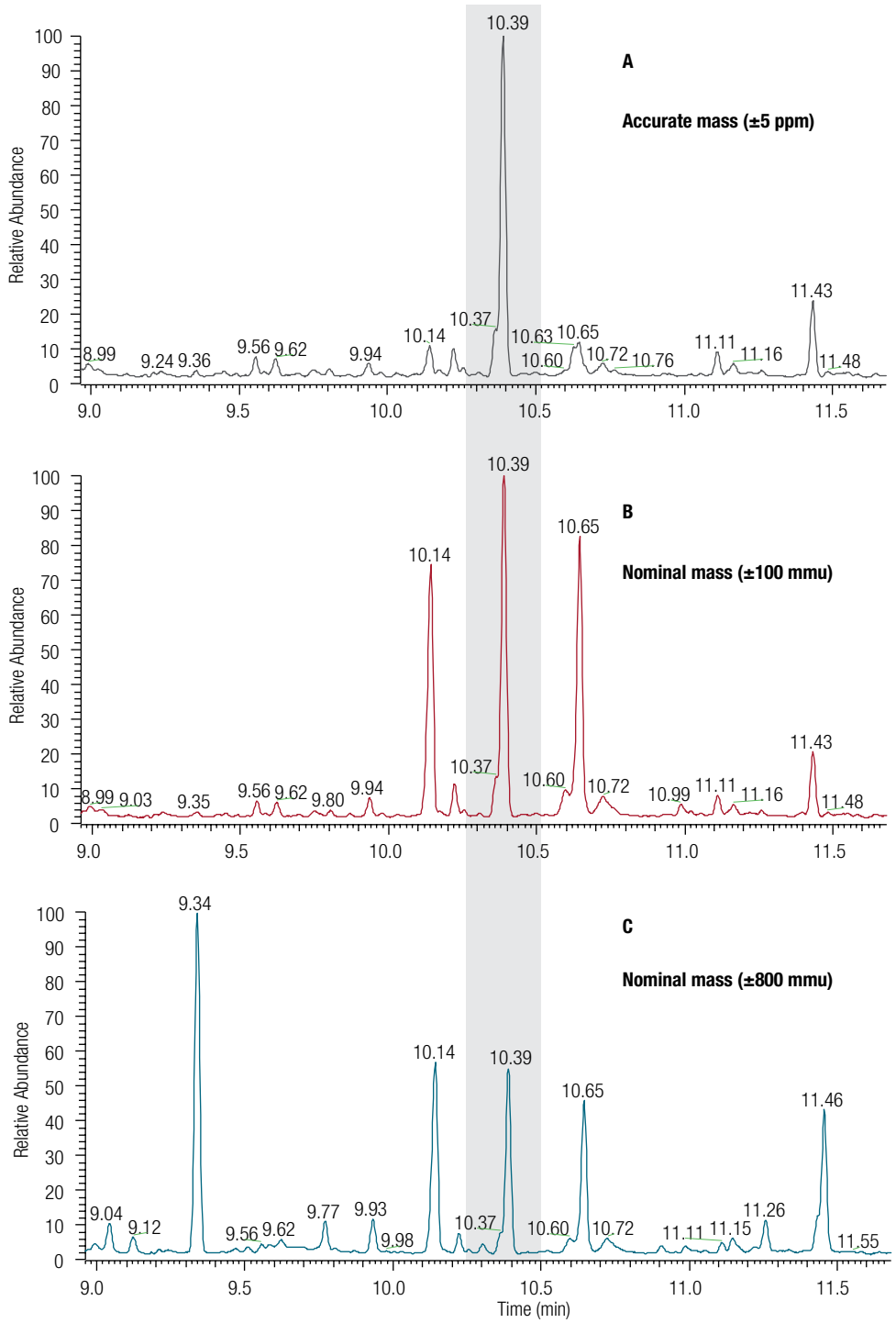

Figure 6. Full-scan accurate mass selectivity demonstrated for PS in a mixed sample containing PS, PA, PC, PE, PMMA, PP, PVC, and PET. Accurate mass measurements enable confident detection (±5 ppm, A), whereas at nominal mass acquisitions additional interfering compounds can be detected (±100 mmu, B and ±800 mmu C).

Additional non-targeted unknown compounds identification

In addition to the targeted quantification, TraceFinder software allows for untargeted analysis of samples that were acquired on GC-Orbitrap systems. This represents a distinct advantage of Orbitrap GC technology due to its routine full-scan, high-resolution mode of operation. This way the analyst can screen the raw data from the quantitation experiment to search for additional chemicals resulting from the pyrolysis process. An example of this is shown in Figure 7 for α-methylstyrene (2-phenylpropene), a known degradation product formed as a consequence of PS pyrolysis[10].

Conclusions

The results of this study demonstrate that:

- The Exactive GC Orbitrap GC-MS system in combination with pyrolysis has proven to be a very promising analytical technique that opens new possibilities with respect to the analysis of microplastic polymers in biological matrices.
- The Exactive GC Orbitrap GC-MS system demonstrates excellent linear response over a concentration range of 0.05 µg to 50 µg absolute weight for each plastic material with accurate quantitative estimation of microplastic polymers in real samples.
- The high resolving power of the Exactive GC Orbitrap GC-MS system facilitates sub-ppm mass accuracy at low and high concentrations, essential for achieving enough selectivity to confidently separate and identify pyrolysis products and reduces detection limits (ex: PS and PP).
- Full-scan acquisition enables the detection and identification of additional compounds produced during the pyrolysis process of microplastics. Putative identifications require confirmation using analytical standards.

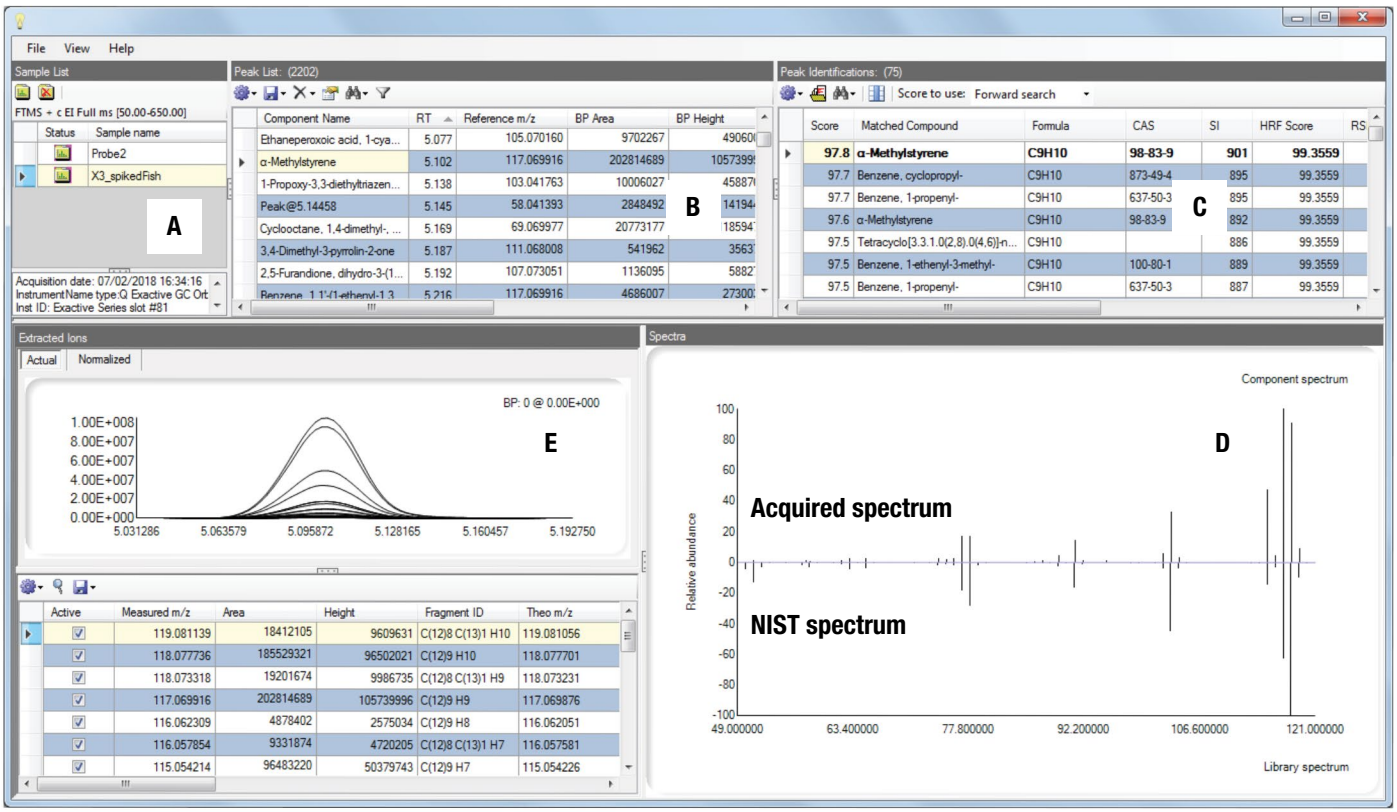

Figure 7. TraceFinder deconvolution browser showing α-methylstyrene (RT=5.1 min) tentative identification based on library (NIST) match (reverse search index, SI 901), fragment rationalization with a confidence score > 97% and mass accuracies of measured fragments (e.g., base peak *m/z* 117.069, Δ_{ppm} = 0.3). Samples processed (A), peaks detected (B), identified chemicals (C), acquired versus library spectra (D), and deconvoluted mass spectra for α-methylstyrene (E) are indicated.

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APPLICATION NOTE

No. 10530

Low level quantification of NDMA and non-targeted contaminants screening in drinking water using GC Orbitrap mass spectrometry

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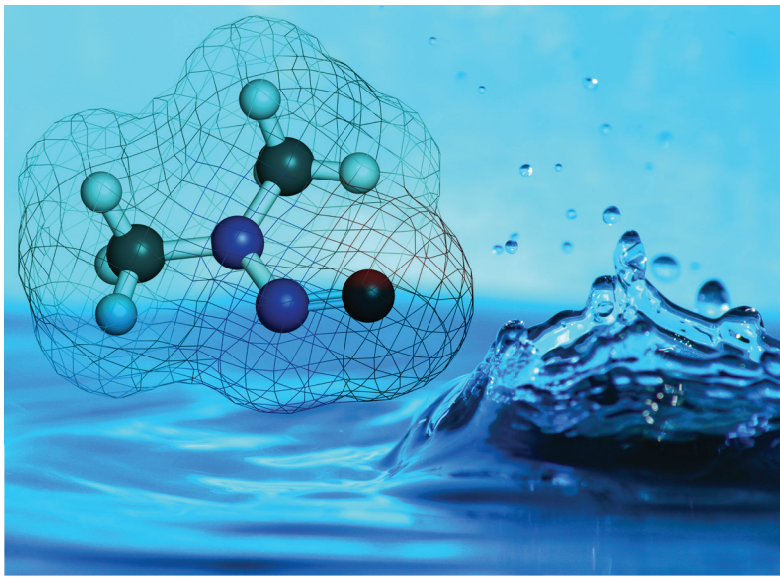
Keywords: N-nitrosodimethylamine, Drinking water, High resolution GC-MS, Exact mass, Non-targeted screening, Quantification, GC Orbitrap, TraceFinder, Environmental

Introduction

N-nitrosodimethylamine (NDMA) is a semivolatile organic compound that belongs to nitrosamines, an emerging class of drinking water contaminants. NDMA is the main nitrosamine of concern and is classified as a potent carcinogen by the U.S. Environmental Protection Agency as it is known to induce tumors following administration by either ingestion or inhalation.[1] NDMA is formed as a by-product during industrial processes such as chloramination of wastewater and drinking water.[2] NDMA is considered a priority pollutant and various countries around the world have already introduced maximum acceptable concentrations of 9 ng/L[6] and action levels 10 ng/L.[7] It is particularly important that NDMA is detected and accurately quantified in drinking water as even low level of this chemical (e.g., 10 ng/L) can pose human cancer risks, being especially toxic to the liver.[1]



Traditionally, the analytical methodology used for NDMA detection and quantification employs single or triple quadrupole gas chromatography mass spectrometry (GC-MS), or magnetic sectors and high resolution time-of-flight mass spectrometers. However, with these analytical instruments it is difficult to obtain high selectivity and high sensitivity at the same time. Reduced selectivity can lead to interferences with the matrix and background chemical ions and can result in false positive detection and erroneous quantification of NDMA.[8] This is due to poor selectivity through insufficient resolving power of such instrumentation.

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In this work, a sensitive and selective method for NDMA detection and quantification using high resolution accurate mass GC Orbitrap technology is described. Test samples were subjected to GC-MS analysis using a Thermo Scientific™ Exactive™ GC Orbitrap mass spectrometer and the quantitative performance of this novel analytical platform was evaluated for sensitivity, mass accuracy, repeatability and linearity of response. In addition to targeted quantification of NDMA, acquiring the data using full-scan high resolution mode allowed for additional contaminants screening and identification in the drinking water samples without the need for separate sample injections or complicated experimental setup.

Experimental conditions

In the experiments described below, an Exactive GC Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD TG-1701MS, 30m × 0.25 mm × 0.25 µm film capillary column (P/N: 26090-1420). 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 (µL):	2.0
Liner:	Single gooseneck (P/N 4530924-UI)
Inlet (°C):	220
Inlet Module and Mode:	Split/Splitless: Surged Splitless
Surge Pressure (kPa):	385
Surge Duration (min):	1.0
Split Flow (mL/min):	80
Column Flow (mL/min)	1.5
Oven Temperature Program:	
Temperature 1 (°C):	35
Hold Time (min):	1
Temperature 2 (°C):	130
Rate (°C/min):	25
Temperature 3 (°C):	230
Rate (°C/min):	125
Hold Time (min):	6

Automated optimization of ion detection and mass calibration was done using perfluorotributylamine (PFTBA) to achieve mass accuracy of <0.5 ppm in <5 minutes. To ensure sufficient selectivity, data was acquired using 60,000 resolving power measured at Full Width at Half Maxima (FWHM) and at *m/z* 200 (Table 2). This is particularly important when NDMA detection is in matrices that contain a high chemical background that can potentially interfere with NDMA ions. These GC-MS settings ensured that chromatographic data was acquired with a minimum of 12 points/peak for consistent peak integration.

Table 2. Mass spectrometer conditions.

Exactive GC System Parameters	
Transfer Line (°C):	260
Ionization Type:	EI
Ion Source (°C):	230
Electron Energy (eV):	70
Acquisition Mode:	Full-scan
Mass Range (*m/z*):	50–650
Mass Resolution (FWHM at *m/z* 200):	60,000
Lockmass (*m/z*):	207.03235

Samples

NDMA analysis involves a solid phase extraction (SPE) of the water samples that concentrate the extracts by a factor of 1000.[3] Taking this into account, the quantification performance of the Exactive GC-MS was tested using both solvent standards and real drinking water (tap water) samples.

The solvent standards were prepared in dichloromethane (DCM) and were spiked with native NDMA in DCM in a similar manner as for real water samples. The final concentration levels in the standards were: 0.1, 1, 10 and 100 µg/L (ppb). Each solvent standard was spiked with 20 µg/L deuterated NDMA (d_6-NDMA) in DCM which was used as an internal standard. In addition to these calibration standards, a procedural blank (DCM not spiked) was used (Table 3).

To validate the results from the solvent standard experiment, three drinking water samples (M1, M5 and M10) were collected in duplicate from the local ICRA facility and spiked with native NDMA prior to SPE extraction at three concentration levels: 0.96, 4.8 and 9.6 ng/L. In addition to these, a drinking water sample that was not spiked with NDMA (M0) was used as matrix blank. Each water sample was subjected to individual SPE extraction (EPA 521/522, Restek) followed by a concentration step

Table 3. Sample preparation for two separate experiments: top table details the preparation of solvent standards used for testing linearity, sensitivity, peak area repeatability; bottom table shows the solvent standards and drinking water samples used to validate the method for NDMA quantification.

Calibration standard	Working solution NDMA (µg/L)	Volume	NDMA conc. (µg/L)	d_6-NDMA 5 ppm (mg/L) added prior to adjusting the volume in the 10 mL flask	Final conc. (µg/L) d_6-NDMA
Cal 6	5000	200 µl in 10 mL flask (DCM)	100	40 µl	20
Cal 5	5000	20 µl in 10 mL flask (DCM)	10	40 µl	20
Cal 4	5	2000 µl in 10 mL flask (DCM)	1	40 µl	20
Cal 3	5	200 µl in 10 mL flask (DCM)	0.1	40 µl	20
Cal 1	0	Blank no NDMA added (DCM)	0	40 µl	20

	NDMA concentration (µg/L)	d_6-NDMA (µg/L)	d_{14}-NDPA (µg/L)
Solvent standards in DCM	0.0	0.0	24.0
Cal 0	0.0	0.0	24.0
Cal 0.1	0.1	0.1	24.0
Cal 1	1.0	1.1	24.0
Cal 2	1.9	2.2	24.0
Cal 10	9.6	10.9	24.0
Cal 20	19.3	21.7	24.0
Cal 50	48.1	54.3	24.0
Tap water samples	**NDMA spiked prior to SPE (ng/L)**	**d_6-NDMA (µg/L)**	**d_{14}-NDPA (µg/L)**
M0A (blank tap water)	0.0	24.0	24.0
M0B (blank tap water)	0.0	24.0	24.0
M1A	1.0	24.0	24.0
M1B	1.0	24.0	24.0
M5A	4.8	24.0	24.0
M5B	4.8	24.0	24.0
M10A	9.6	24.0	24.0
M10B	9.6	24.0	24.0

to a 1.0 mL final volume in accordance to the EPA 521 method.[3] To correct for recovery, d_6-NDMA was spiked prior to SPE in each of the tap water samples at 24 ng/L level and used as a surrogate. Also, to correct for sample injection, d_{14}-N-nitrosodipropylamine (d_{14}-NDPA) was used as an internal standard and was added to the final 1.0 mL extract in each sample and standard at 24 pg/µL level.

Data processing

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software which allows for easy set-up and complete quantitative and qualitative analysis workflows. This includes peak integration, calculation of compound concentration and recoveries as well as easy data review and reporting. In addition, for qualitative analysis, TraceFinder automatically generates clean mass spectra following automated peak deconvolution and, compound identification (by searching a custom made, NIST compatible accurate mass library and commercially available spectral libraries).

Results and discussion

The objective of the analysis was to assess the use of GC Orbitrap technology for the analysis of NDMA in drinking water samples at a very low concentration level and for a broad scope non-targeted screening of the samples for the detection and identification of additional contaminants.

NDMA chromatography, sensitivity, linearity and peak area repeatability were evaluated using solvent based standards. This was followed by validation of the method using drinking water samples that were spiked with NDMA at low levels prior to SPE extraction and concentration. In addition to NDMA quantification, the water samples were also screened, using a non-targeted approach, for the presence of additional chemical contaminants. Putative identifications based on NIST library matches, fragment ion rationalization and accurate mass information were made.[4]

Chromatography and resolution

Using the GC conditions stated in the Table 1, fast GC separation (total GC run time 11 min), with good chromatographic separation was obtained, allowing for a high sample throughput. An example of chromatography for NDMA in the lowest calibration solvent standard (0.1 µg/L) and in the lowest level spiked drinking water sample (0.96 ng/L) is shown in Figure 1.

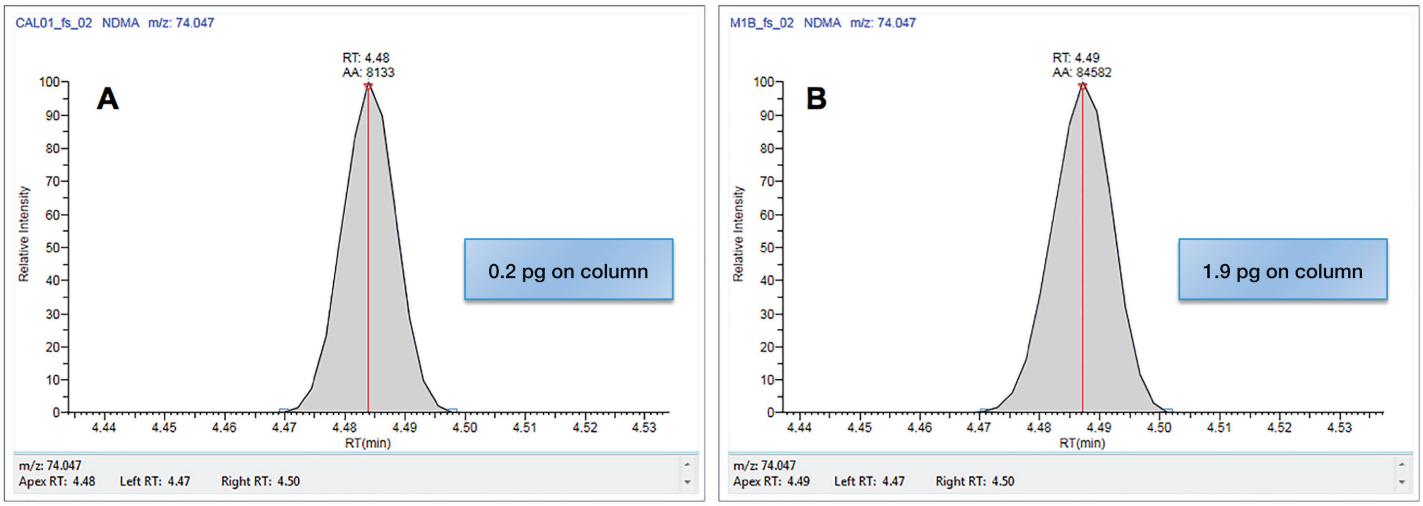
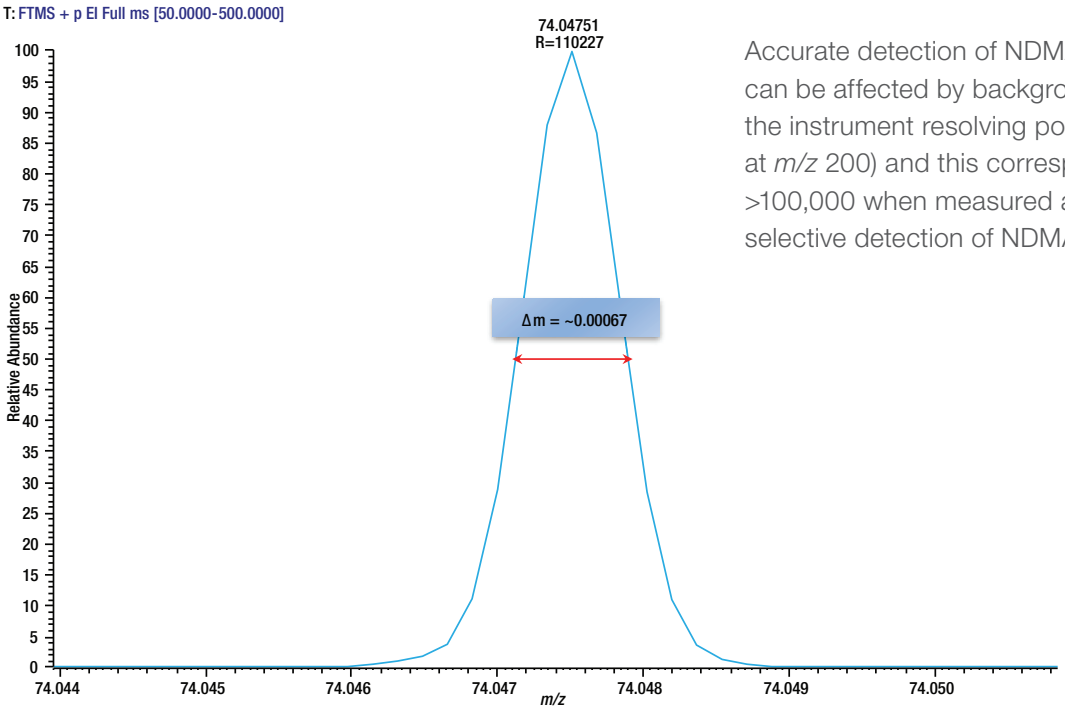


Figure 1. Extracted ion chromatogram (XIC, EI at 70eV) of the *m/z* 74.04747 corresponding to NDMA molecular ion at 0.1 µg/L in the lowest calibration solvent standard (A) and at 1.0 ng/L in a drinking water sample (B). The absolute amount on column is shown as pg of NDMA on column.



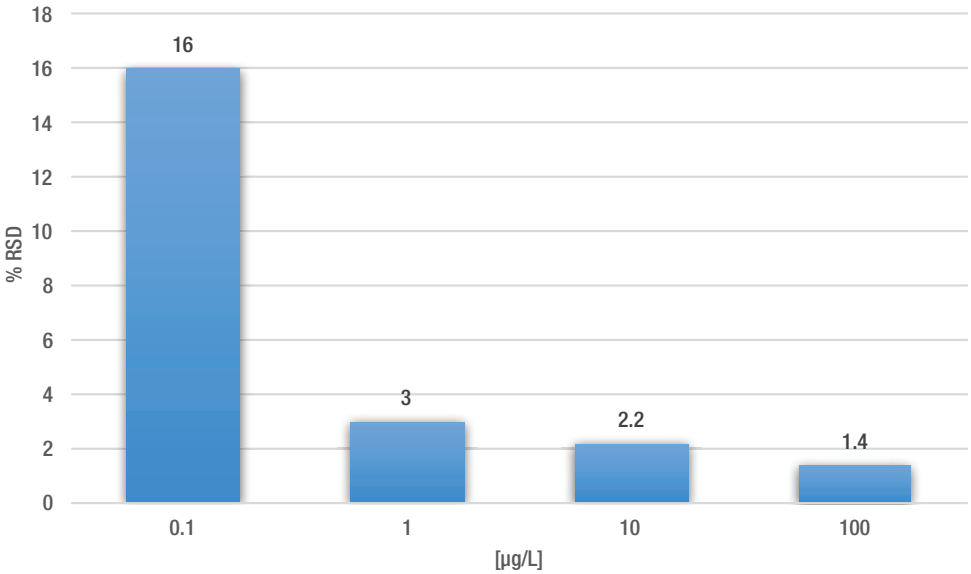
Accurate detection of NDMA molecular ion (*m/z* 74.04747) can be affected by background ions. In all experiments, the instrument resolving power was set to 60,000 (FWHM at *m/z* 200) and this corresponded to a mass resolution of >100,000 when measured at *m/z* 74, sufficient to achieve selective detection of NDMA target ions in matrix (Figure 2).

Figure 2. NDMA in a drinking water sample showing a mass resolution R> 110,000 (FWHM) measured at *m/z* 74.04747. Data acquired in full-scan using electron ionization at 70 eV.

Estimated Instrument Detection Limit (IDL) and peak area repeatability

System sensitivity was assessed by calculating the minimum quantifiable limit or the instrument detection limit (IDL) for NDMA. This was done by using the peak area %RSD derived from n=9 repeat injections of the lowest calibration standard 0.1 µg/L and taking into account the Student's-t critical values for the corresponding degrees of freedom (at 99% confidence). The results of this experiment showed that IDL derived from the Exactive GC system data was 0.09 µg/L, a value similar to the lowest calibration standard detectable.

For reliable quantification robust instrumental response is important, and this was demonstrated by assessing the peak area repeatability of NDMA quantification ion (*m/z* 74.04747). To achieve this, each solvent standard was injected five times except for the 1.0 µg/L standard which was injected nine times. Absolute peak area repeatability was evaluated by looking at %RSD at each concentration level and the results obtained are shown in Figure 3 below.



*n=5 injections per calibration standard were used except 1.0 µg/L level; where n=9 inj. were used

Figure 3. Absolute peak area repeatability of NDMA at various concentration levels (n=5 for 0.1, 10 and 100 µg/L and n=9 for 1.0 µg/L). The average %RSD values at each level are indicated above each bar.

Excellent peak area repeatability was demonstrated using the two batches of samples. For the experiment using solvent standards, across a total number of injections of n=35 the %RSD calculated from the peak area response of the d_6-NDMA internal standard was <4.5%, whereas the %RSD peak area response of the d_{14}-NDPA across n=14 injections (including water samples) was ~5% (Figure 4).

Mass accuracy

It is well known that analyte selectivity increases with higher mass accuracy, therefore obtaining consistent sub ppm accurate mass information provides distinct advantages in complex matrices and for the confident characterization and confirmation of a target chemical. In this study, the mass accuracy for NDMA *m/z* 74.04747 was always <1 ppm at low and high levels in both solvent standards and in extracted drinking water samples (Figure 5 and Table 4).

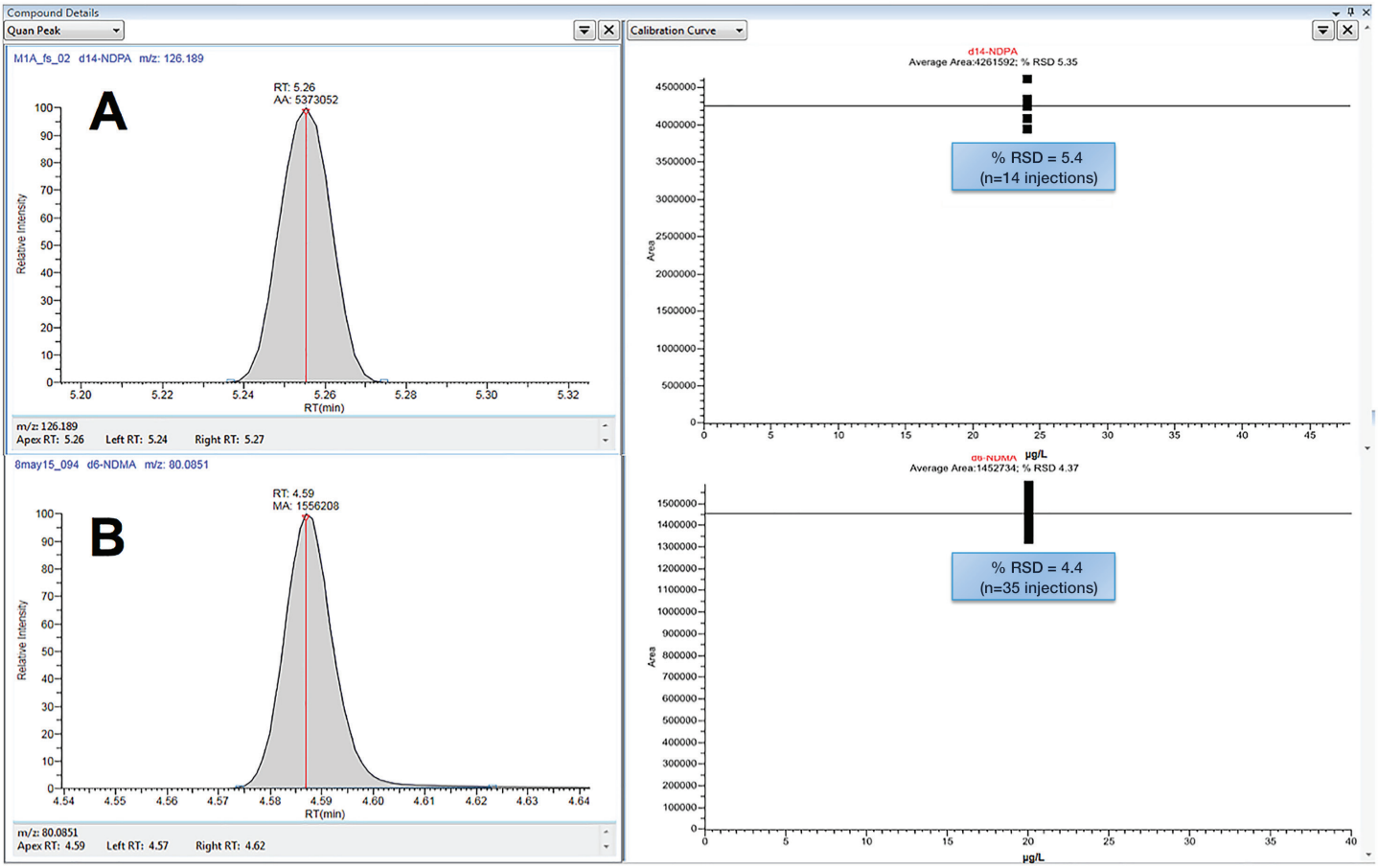


Figure 4. Peak area repeatability (as %RSD) demonstrated for two internal standards corresponding to two different experimental batches: d_{14}-NDPA internal standard across n= 14 injections (A), and d_6-NDMA internal standard across n= 35 injections (B).

Linearity of response

Quantitative linearity was assessed across a 7-point concentration range of 0.1, 1.0, 2.0, 10, 20 and 50 µg/L (ppb) including a solvent blank, each injected twice. Calibration linearity, assessed using a 1/x weighted linear regression, showed that the coefficient of determination (R^2) was >0.999 (Figure 6). Moreover, the %RSD of the relative response factors (RRF) for native NDMA was <9% and for its corresponding d_6-NDMA surrogate the %RSD RF was 6% (Figure 6).

Quantification of NDMA in drinking water samples

Calculated NDMA concentrations in the drinking water samples show good accuracy of the method (Table 4). Surrogate d_6-NDMA recovery was monitored throughout the entire sample sequence with the recovery values obtained in very good agreement with the method 521, which requires that surrogate recovery should be within 70–130% (Table 4). Overall, these results indicate that the Exactive GC mass spectrometer delivers excellent results and is highly suitable for routine laboratory use.

Table 4. Quantification results in drinking water samples

Sample	% recovery d_6-NDMA	Calculated NDMA concentration (ng/L)	Mass error [ppm]
M1A	107	1.1	0.7
M1B	105	0.96	0.01
M5A	111	4.7	0.01
M5B	104	4.3	0.01
M10a	88	8.4	0.2
M10b	99	8.1	0.1

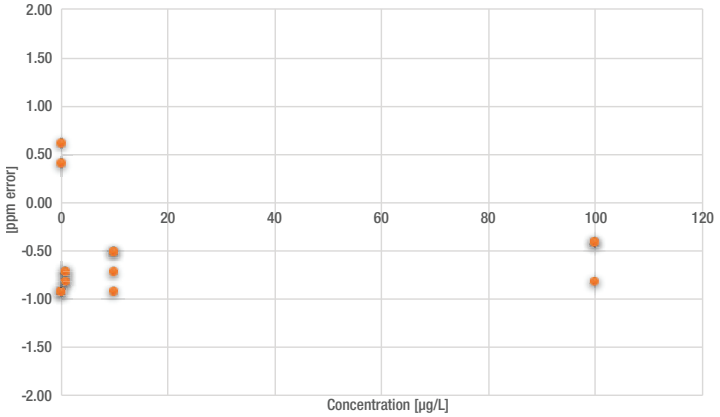


Figure 5. Mass accuracy measurements for NDMA quantitation ion *m/z* 74.04747. NDMA concentration levels (µg/L on the X-axis) as well as the corresponding mass error (ppm Y-axis) are shown. Each dot represents a separate injection.

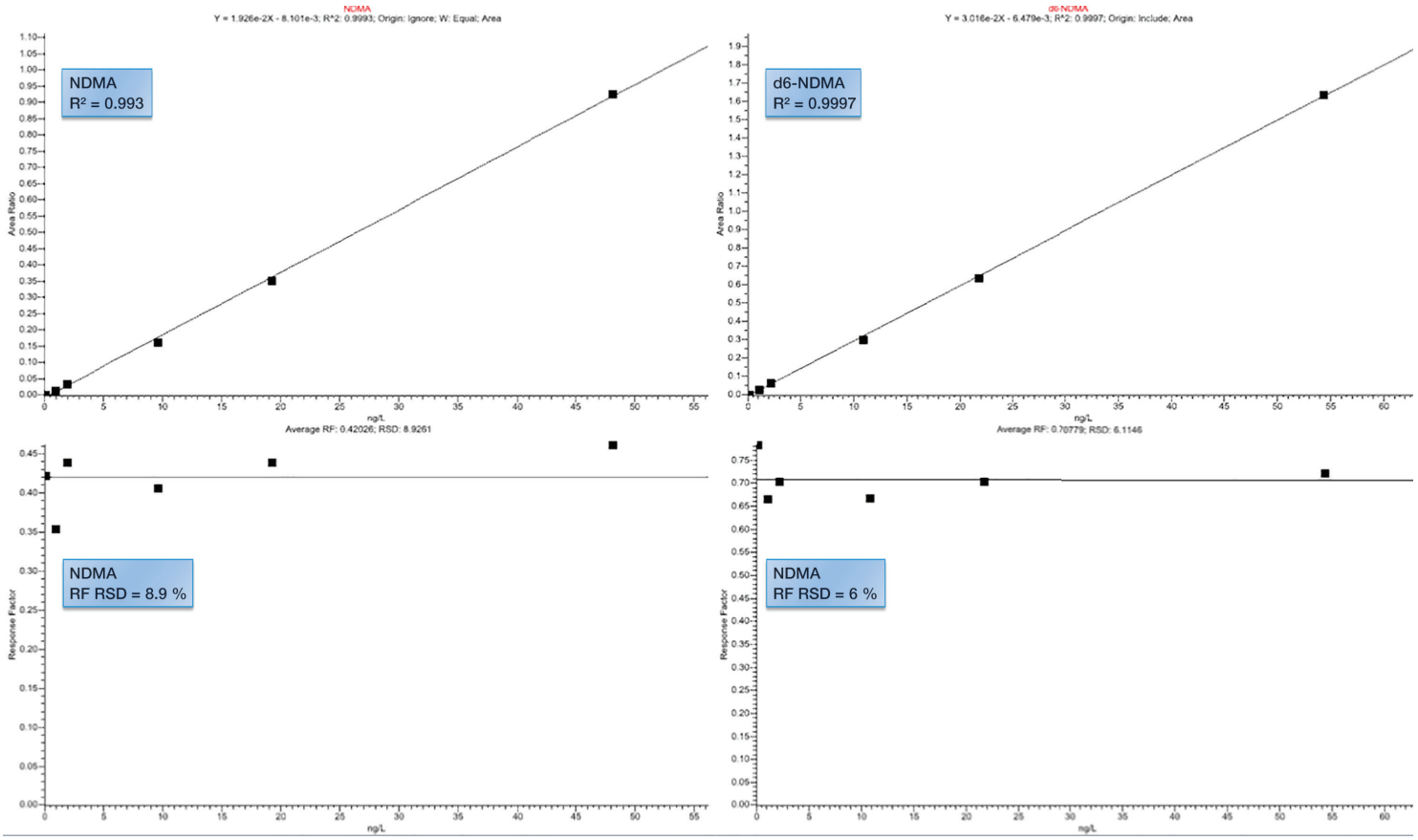


Figure 6. Linearity of NDMA (left) and d_6-NDMA (right) internal standard corrected with d_{14}-NDPA over a 7-point calibration curve (0.1-50 µg/L) showing the corresponding %RSD RF <9% for NDMA and 6% for the d_6-NDMA surrogate.

Non-targeted screening of drinking water samples for additional contaminants

A significant advantage of Exactive GC technology is that, due to its full-scan high resolution mode of operation, the analyst can screen the raw data used for the quantitation experiment for additional, potentially harmful chemical contaminants. This was demonstrated in this work using the data acquired from the drinking water samples which was subjected to a non-targeted screening workflow with TraceFinder. This workflow automates compound deconvolution to obtain clean ion spectra that is then submitted to a library search for putative compound identification. A detailed description of this workflow is described elsewhere.[4]

The results of this data processing show that the drinking water samples contain 220 additional chemicals not present in the procedural DCM solvent blank. These compounds were putatively identified using NIST library (using a forward search index threshold of 800) and a high resolution filtering score (HRF) threshold of 80. The HRF uses the accurate mass information to explain a NIST (or similar) library matched ion spectra.[4] The majority of the contaminants found in the drinking water samples are halogenated organic compounds, pharmaceuticals (ex: Clindamycin, Felbamate), monoterpenes (D-limonene) and phthalates etc. Examples of chemicals detected and identified with high confidence are shown in Figure 7. Chloroiodomethane has been previously reported in the literature as a disinfection by-product.[4] Also, tetrachloroethylene is a widely used as a dry cleaning chemical often found in private or public drinking water and it is known to adversely affect human health.[5] Both chemicals were identified with an excellent library match (SI >890), a total score >95% and a mass accuracy for the molecular ions <0.5 ppm.

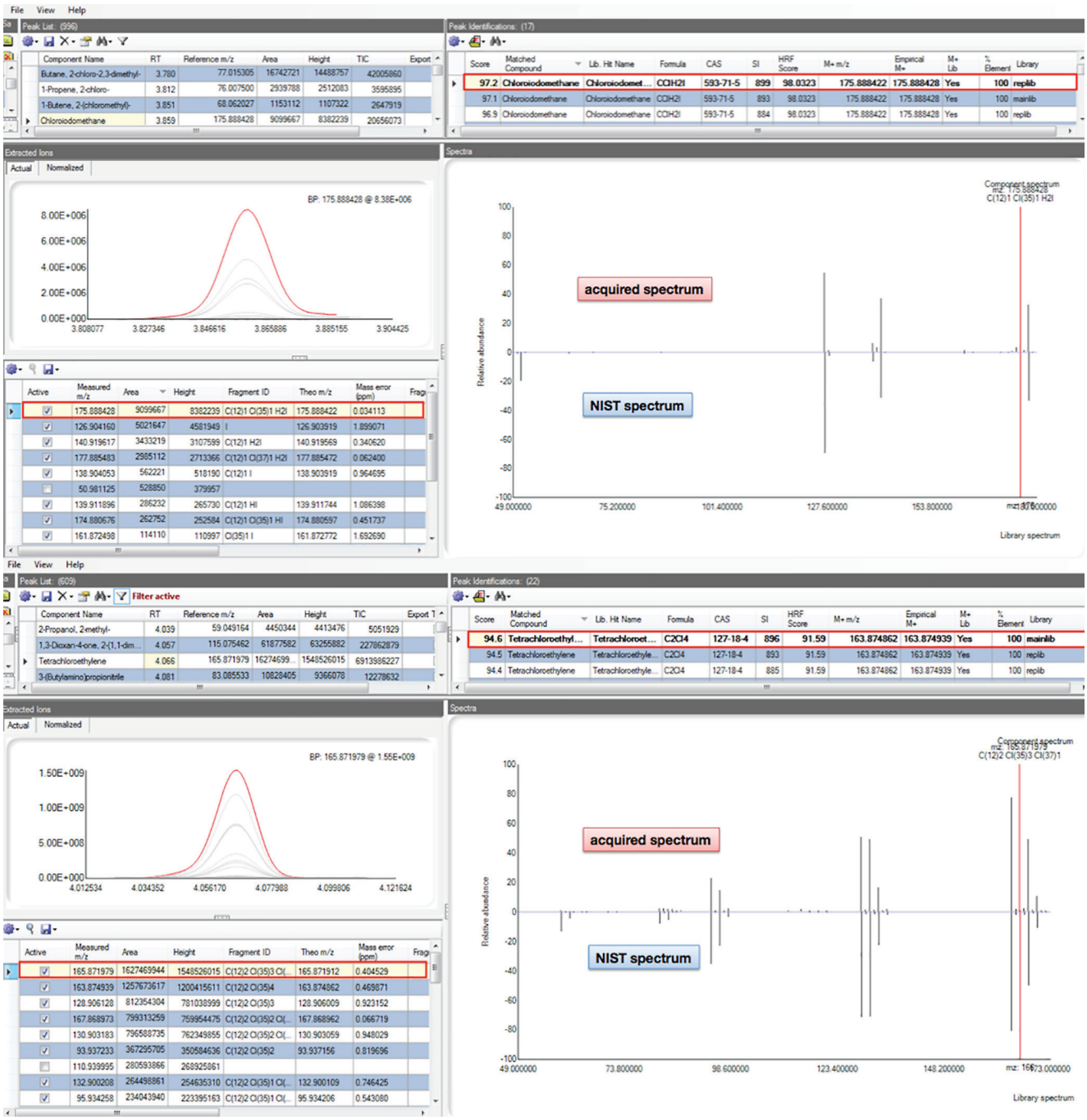


Figure 7. Examples of chemical contaminants present in the drinking water sample. TraceFinder deconvolution browser highlighting chloroiodomethane (a) and tetrachloroethylene (b) with their corresponding deconvoluted mass spectra, the total identification confidence score, NIST library match (forward SI) and accurate mass measurements for each of the measured ions.

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Conclusions

With the Exactive GC system in full-scan operation at 60,000 resolution (FWHM), NDMA was detected at 0.1 µg/L level in the lowest calibration level, which, assuming 100% recovery, will translate to an NDMA limit of detection (LOD) of 0.1 ng/L. In addition, NDMA was easily detected and accurately quantified at 1.0 ng/L in the drinking water samples with excellent recovery values.

Full-scan acquisition enabled the detection and putative identification of additional harmful contaminants in the drinking water samples. Halogenic organic compounds were predominantly detected and their presence is most probably related to the disinfection processes that involves chloramination and chlorination reactions. Putative identifications require further confirmation using analytical standards.

In addition to very high sensitivity, excellent linear response across 0.1–50 µg/L was observed for both NDMA (R^2 >0.999 and residuals <9 %RSD RF) and for its corresponding d^6-NDMA surrogate (R^2 >0.999 and residuals <6 %RSD RF).

Moreover, consistently low (sub ppm) mass deviation from the theoretical NDMA mass was observed at all concentration levels and in all analyzed samples.

Taken together, these results described in this study demonstrate excellent quantitative and qualitative performance of the Exactive GC system for the analysis of trace levels of NDMA.

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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

Authors

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Keywords

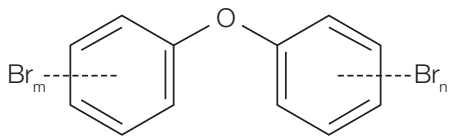
Polybrominated diphenyl ethers, PBDE, high-resolution GC-MS, accurate mass, quantification, GC Orbitrap, environmental, sediment, filter dust, sludge, air

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)

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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.

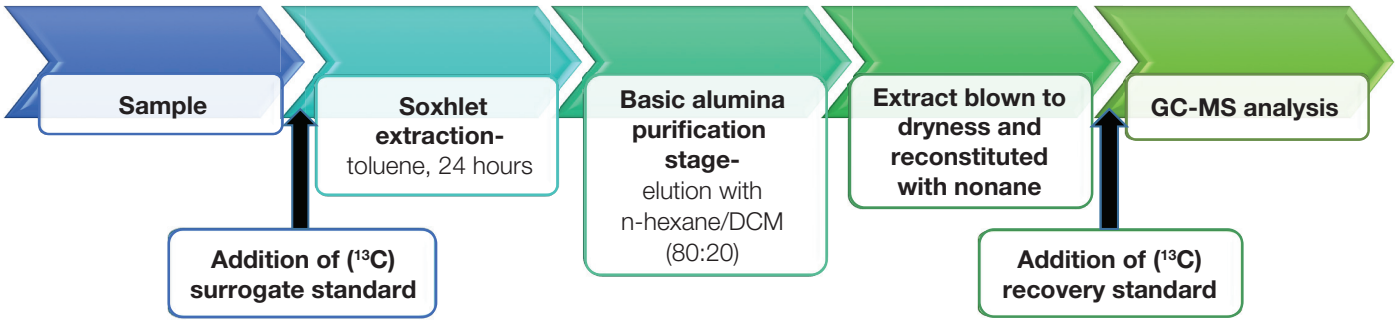


Figure 2. PBDE analytical workflow, including sample extraction, extract purification, and concentration stages required prior to GC-MS analysis

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).

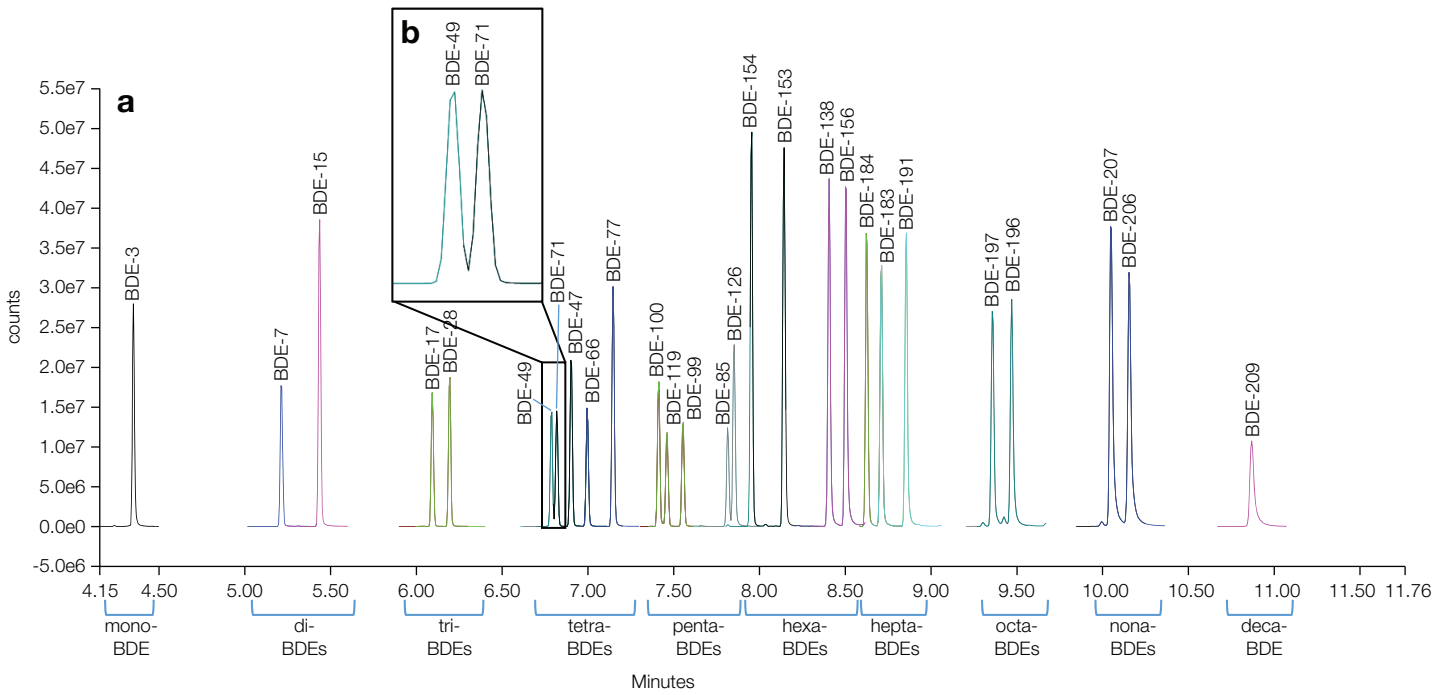


Figure 3. (a) Overlaid extracted ion chromatograms (EICs ±5 ppm extraction window) for the 27 native PBDE congeners in a solvent standard at 400 pg on column for mono- to penta-BDEs, 800 pg on column for hexa- to octa-BDEs, and 2000 pg on column for nona- to deca-BDEs and (b) separation of critical pair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

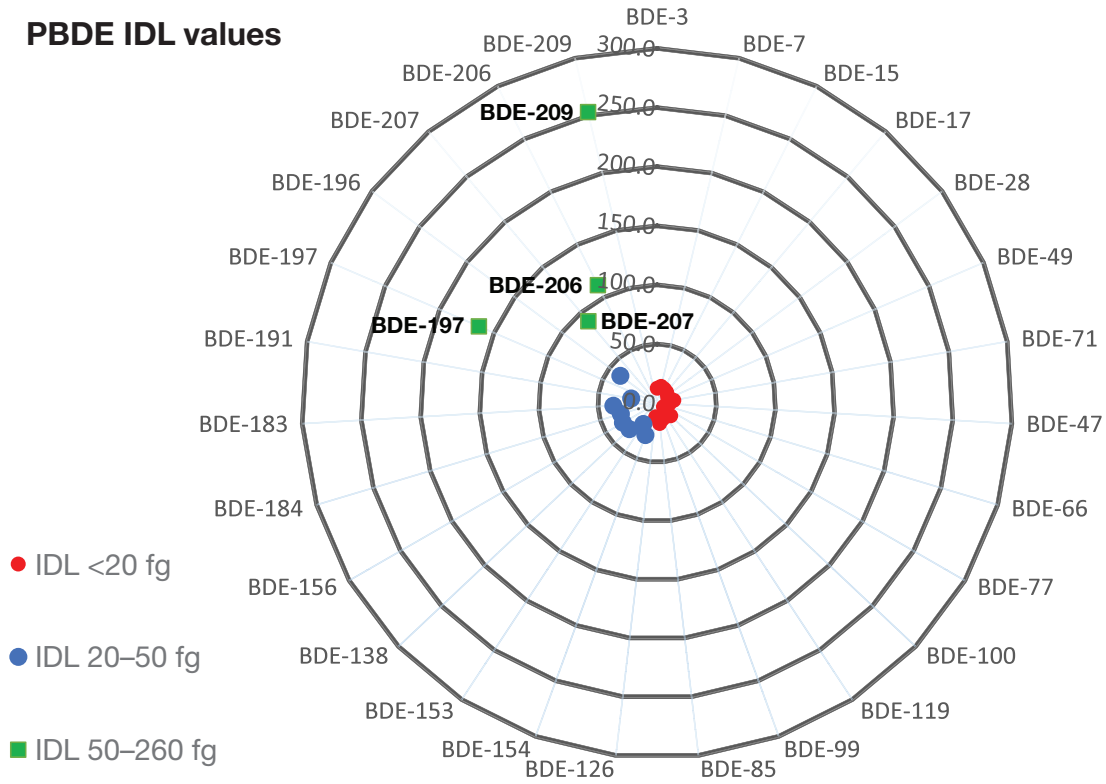


Figure 4. Calculated IDL values for 27 native PBDE congeners, statistically calculated from the results of n=14 replicate injections of the lowest serial diluted solvent standard

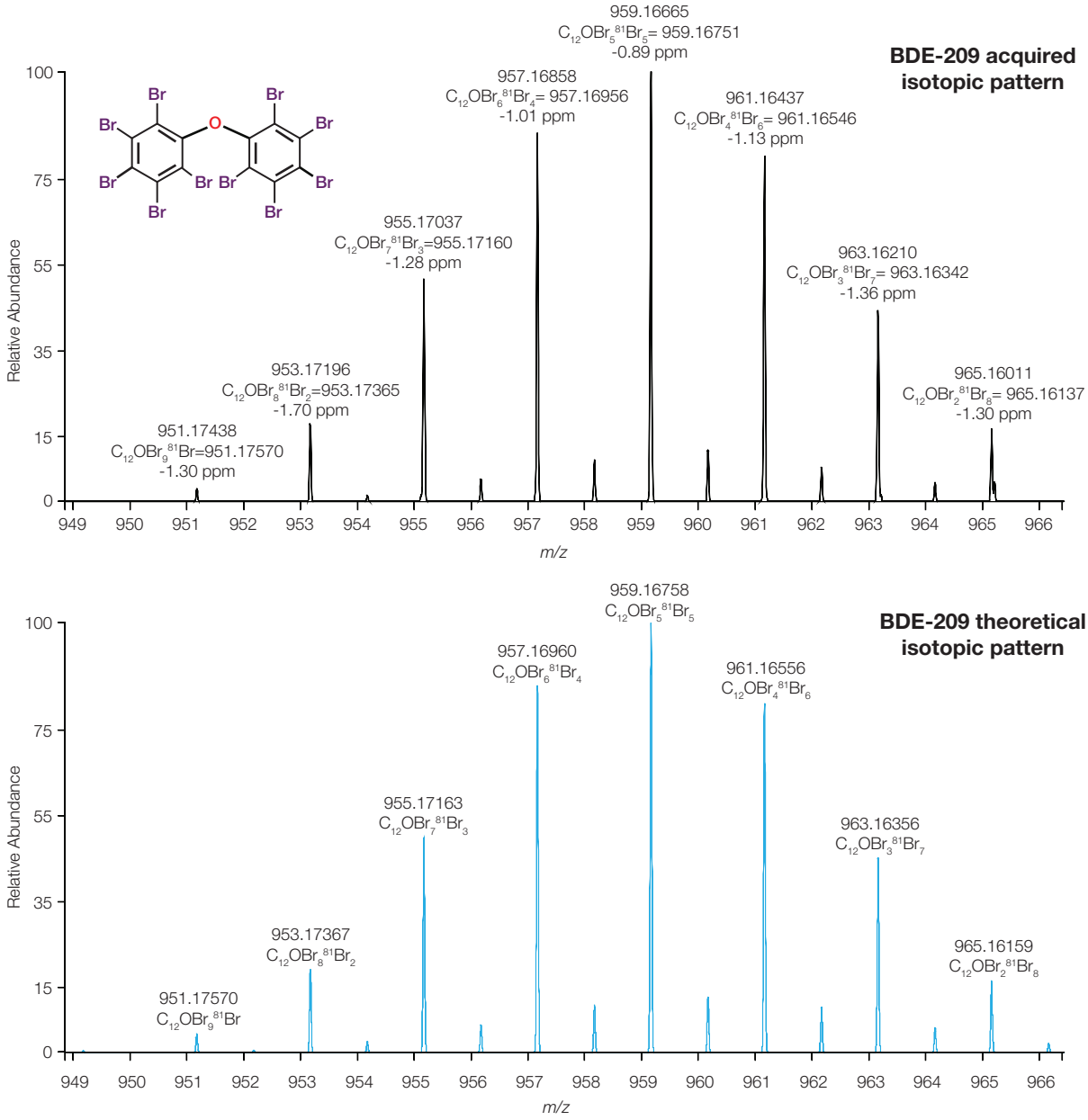
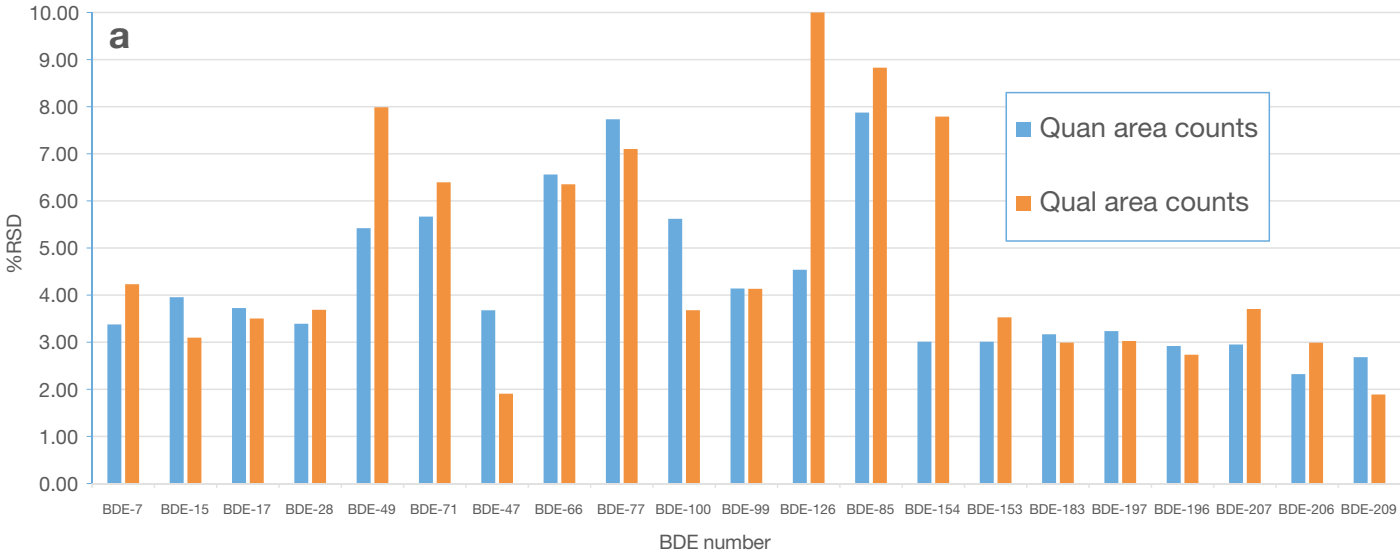


Figure 5. Comparison of mass spectra for BDE-209, acquired isotopic pattern (upper) versus the theoretical isotopic pattern (lower). Consistent <2 ppm mass accuracy obtained for each of the ion in the cluster. Annotated are the measured mass, the elemental composition, and the theoretical mass as well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



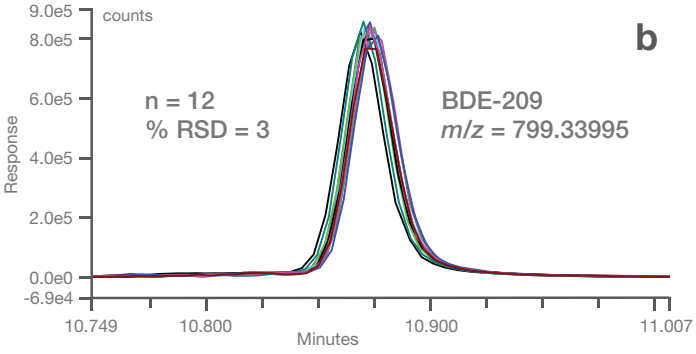


Figure 6. Replicate injections (n=12) of a filter dust sample, a) quantification and qualification area counts %RSD values for identified congeners, b) overlaid EICs (*m/z* 799.33995) for BDE-209

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.

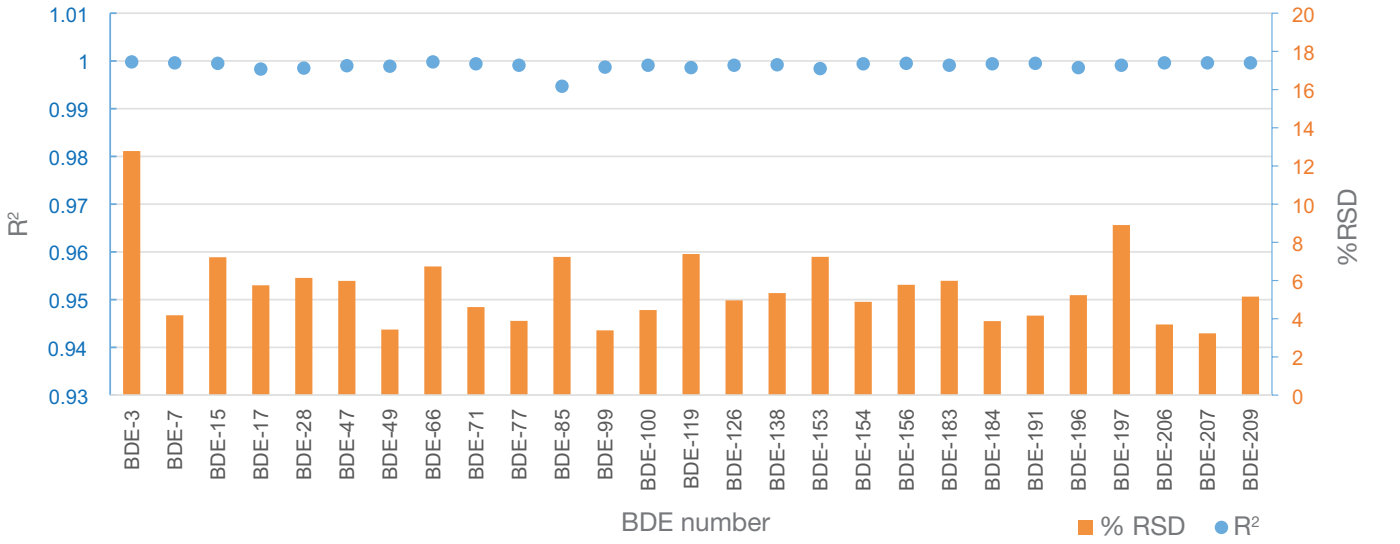


Figure 7. Coefficient of determination (left) and residuals values (%RSD) for 27 native PBDE congeners (right)

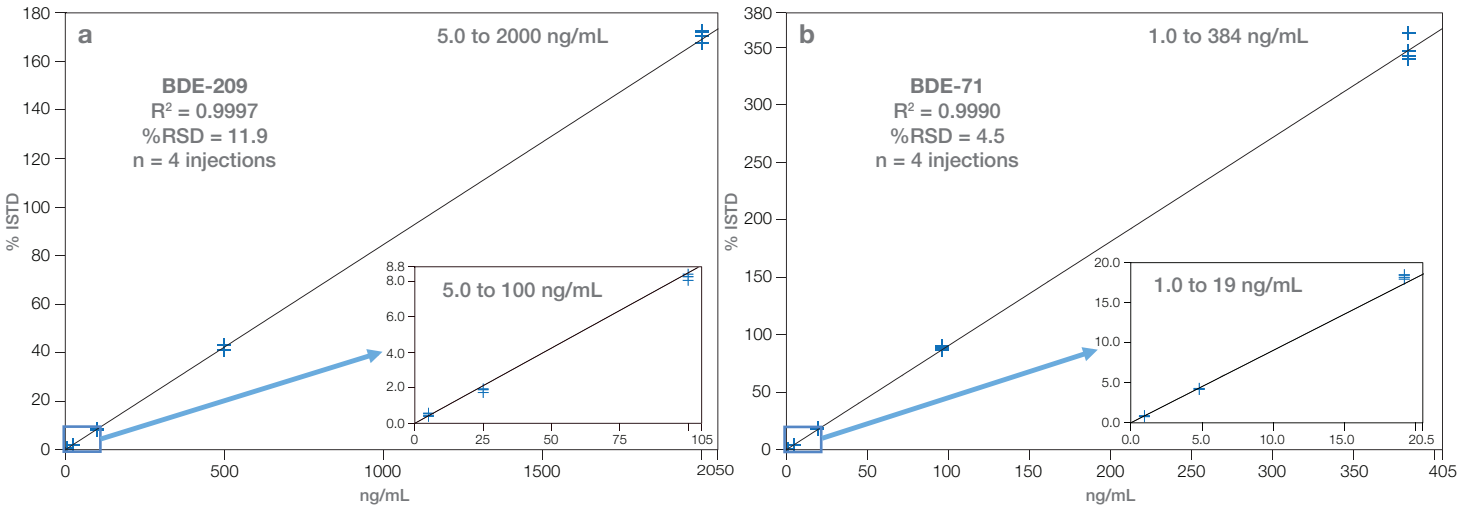


Figure 8. Example calibration curves (a) BDE-209 and (b) BDE-71, illustrating the linearity obtained. The inset calibration curves exemplify the maintained linearity for the lowest 3 calibration levels.

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.

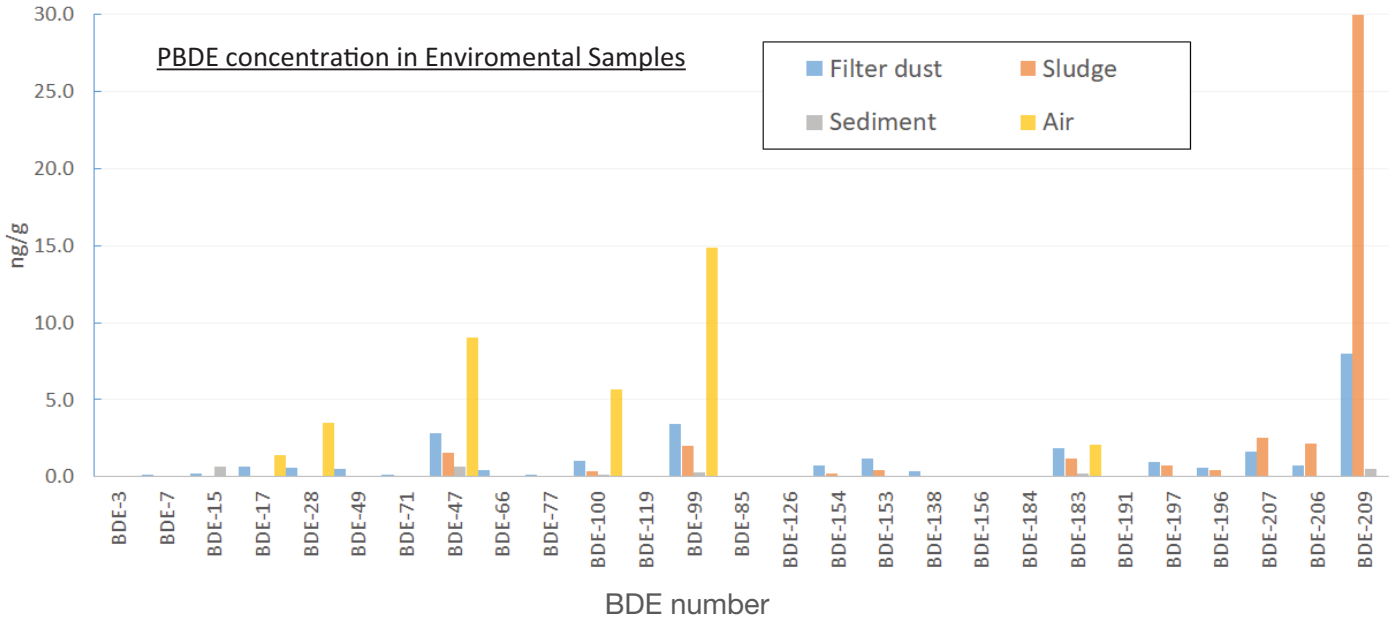


Figure 9. Calculated concentration of PBDEs, extracted and quantified from filter dust, sludge, sediment and air samples, thus illustrating the predominant PBDE congeners identified in the analyzed sludge samples as BDE-209, 206, 207, and 99, filter dust samples as BDE-209, 47, and 99, air samples as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

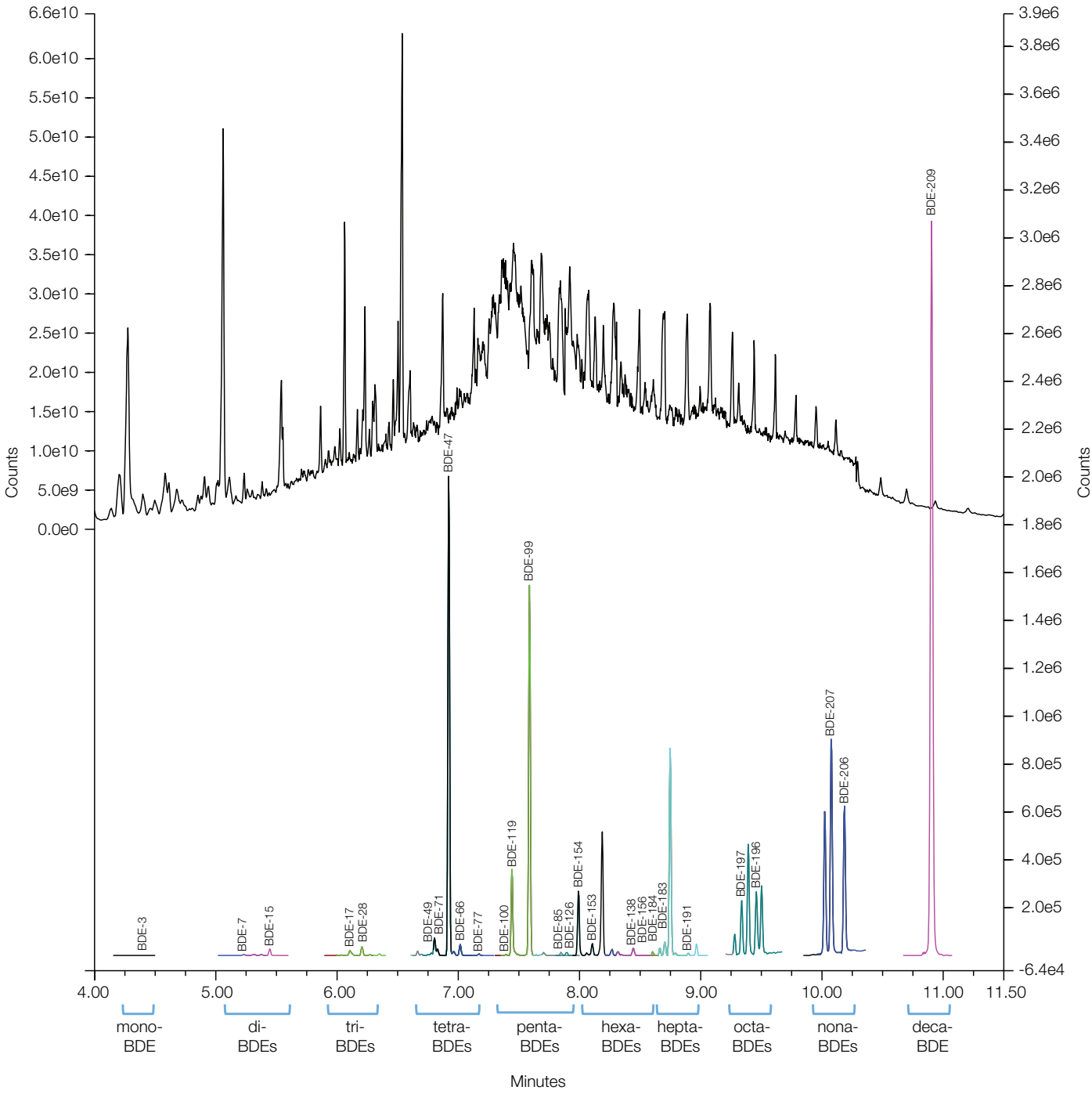


Figure 10. Sludge sample chromatograms: (upper) TIC full scan; (lower) EICs for the native PBDE congeners identified in the sample

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).

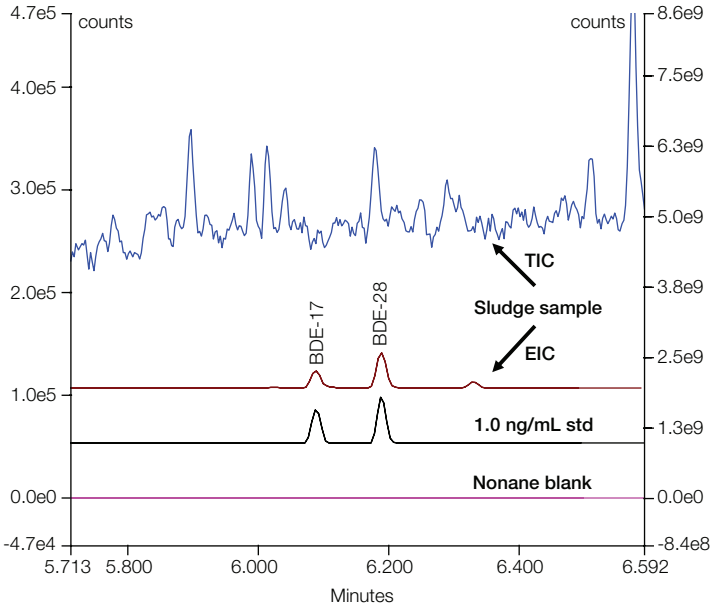


Figure 11. Overlaid EICs for BDE-17 and BDE-28 (left), in 1.0 ng/mL standard, an extracted sludge sample at a similar level, and a nonane blank. In addition, the TIC for the extracted sludge sample (right).

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

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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 10623

Versatility of GC-Orbitrap mass spectrometry for the ultra-trace detection of persistent organic pollutants in penguin blood from Antarctica

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Keywords

Persistent organic pollutants, POPs, polychlorinatd biphenyls, PCBs, organochlorine pesticides, King penguin blood, liquid-liquid extraction, GC Orbitrap high-resolution mass spectrometry, full-scan, targeted single ion monitoring, accurate mass, simultaneous full-scan/targeted analysis acquisition

Goal

In this study, the performance of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ mass spectrometer was evaluated for routine analysis of POPs within King penguin blood from Antarctica.

Introduction

Persistent organic pollutants (POPs) have been studied extensively over the past five decades due to their toxicity and environmental persistence with spread and exposure throughout the global environment. International treaties, such as the Stockholm Convention, effectively banned or restricted the use of POPs such as polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in 2004. However, many of these chemicals are still present in the environment today, accumulating in the food chains and posing risks for biota and humans even when present in trace amounts. PCBs and OC pesticides are the dominant contaminants found in remote polar regions, as these regions act as deposition sinks for contaminants that have undergone long-range transport via atmospheric or oceanic currents.[1] Although Arctic exposure to POPs has been studied extensively, information is scarce within Antarctica. Historically, chemical usage within the Southern Hemisphere has been lower compared to the Northern Hemisphere, making detection of POPs more challenging as concentrations are much lower. However, the Antarctica continent does receive atmospheric inputs of POPs.[2]

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This together with increasing industrial and agricultural activity within the Southern Hemisphere indicates an exposure risk to this region and highlights the need to address analytical challenges associated with monitoring POPs in Antarctica biological matrices.[3]

As environmental concentrations are approaching the detection capabilities of current analytical instrumentation, sample amount and preparation is key. However, sample material obtained through non-invasive sampling (i.e., blood or plasma) is often limited. Employing extensive sample clean-up strategies will increase sample processing time and costs and potentially result in lower detection frequency, as targeted compounds present at low concentrations will be lost through various clean-up steps. In addition, dilution of samples to reduce effects of co-extracted matrix may dilute targeted analytes below detection limits and reduce detection frequency. High-resolution Orbitrap™ mass spectrometry provides distinct advantages to help address these analytical challenges. With the potential to run routine full-scan and/or targeted single ion monitoring (t-SIM) analysis at 60,000 mass resolution (measured at *m/z* 200 as full width half maximum) greatly enhances the ability to selectively target and separate the analytes of interest away from co-extracted matrix interferences, reducing chemical noise and lowering detection limits.

Experimental conditions

Sample preparation

Blood samples from King penguins (0.5–1.0 g wet weight) were spiked with ^{13}C mass-labeled internal standards (PCBs and OC pesticides) and extracted using a liquid-liquid extraction procedure. Then, 2 mL ethanol, 2 mL of deionized water saturated with ammonium sulfate, and 6 mL of hexane were added to the sample material. Samples were vortexed and hexane supernatant was collected. Sample extracts were evaporated to dryness, then reconstituted in 0.5 mL of hexane and cleaned up further using automated solid phase extraction using 1 gram of activated Florisil® (450 °C) and eluted with 12 mL of 10% DCM/hexane. Sample extracts were evaporated close to dryness and quantitatively transferred to a GC autosampler micro-insert vial. ^{13}C-PCB 159 was added as a syringe standard. Isotope dilution quantification of target compounds in the samples was performed taking into account the corresponding internal standard response and using a single point calibration.

Instrument and method setup

Sample extracts were analyzed using the Q Exactive GC Orbitrap mass spectrometer. Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 5 m integrated guard (P/N 26096-1425). Sample analysis was performed using full-scan and t-SIM acquisition. Additional details of instrument parameters are displayed in Table 1 and Table 2.

Table 1. GC and injector conditions

TRACE 1310 GC System Parameters	
Injection volume:	1 µL
Liner:	Thermo Scientific™ LinerGOLD™ GC Liner (P/N 453A1345-UI)
Inlet:	250 °C
Carrier gas:	He, 1.2 mL/min (constant flow)
Oven temperature program	
Temperature 1:	40 °C
Hold time:	1.5 min
Temperature 2:	180 °C
Rate:	25 °C/min
Hold time:	0 min
Temperature 3:	280 °C
Rate:	5 °C/min
Hold time:	0 min
Temperature 3:	320 °C
Rate:	40 °C/min
Hold time:	5 min

Table 2. Mass spectrometer conditions for data acquisition using simultaneous full-scan and targeted single ion monitoring (t-SIM) mode

Q Exactive GC Mass Spectrometer Parameters	
Transfer line:	280 °C
Ionization type:	Electron Ion (EI)
Ion source:	250 °C
Electron energy:	70 eV
Acquisition mode:	Full-scan and t-SIM
Isolation window:	8 Da
Mass range:	50–600 Da
Resolving power:	30,000 & 60,000 (FWHM at *m/z* 200)
Lock mass, column bleed:	207.03235 *m/z*

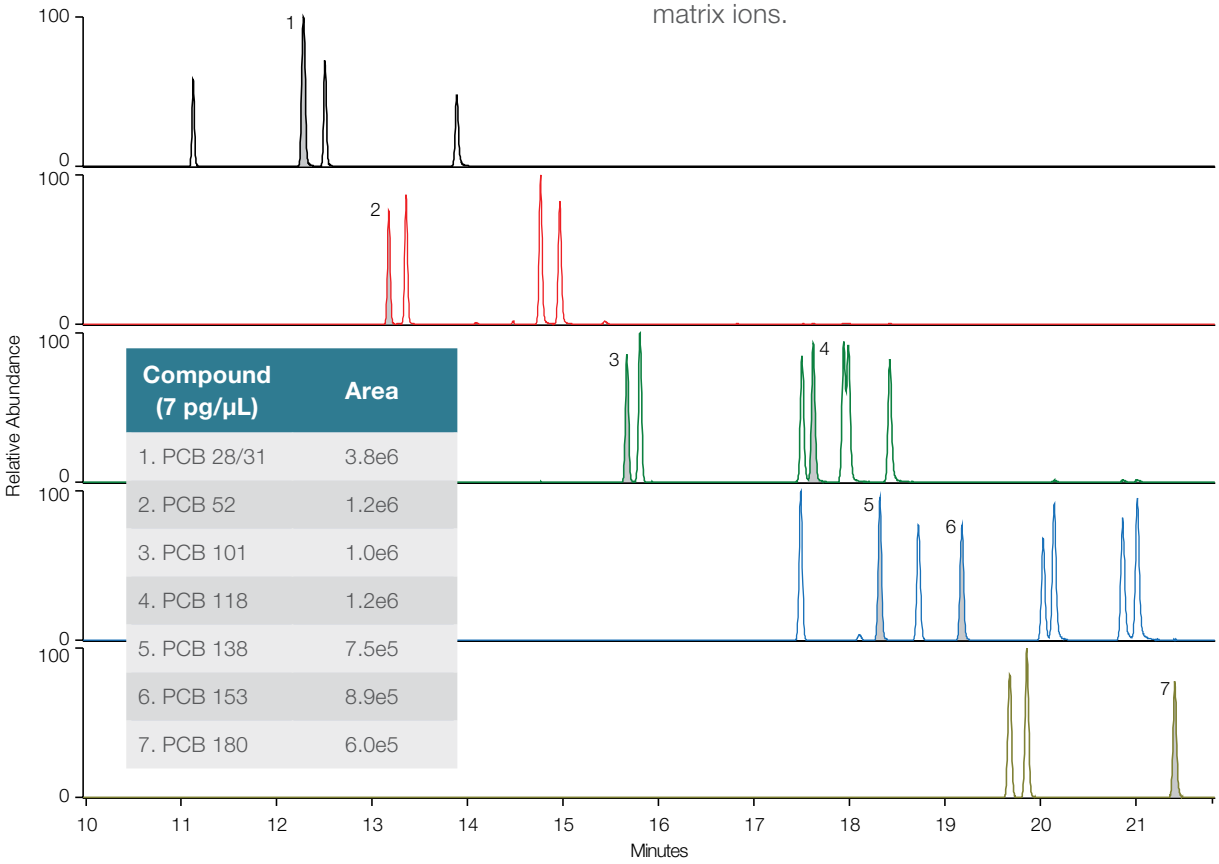
Data processing

Data were acquired using Thermo Scientific™ TraceFinder™ Environmental and Food Safety software version 4.1. Performance of the Q Exactive GC Orbitrap mass spectrometer to measure POPs in King penguin blood was evaluated in both full-scan and t-SIM acquisition modes. For targeted analysis, accurate masses for both target and qualifier ions were automatically acquired from quantification standards using TraceFinder software. Positive detection of compounds required both the target and qualifier ion to be detected within a mass accuracy of ±5 ppm and a target/qualifier ion ratio within 20% of that obtained from the quantification standard. All concentrations were blank corrected (if detected) with 3 and 10 times the blank variation being used to determine limits of detection and quantification, respectively.

Results and discussion

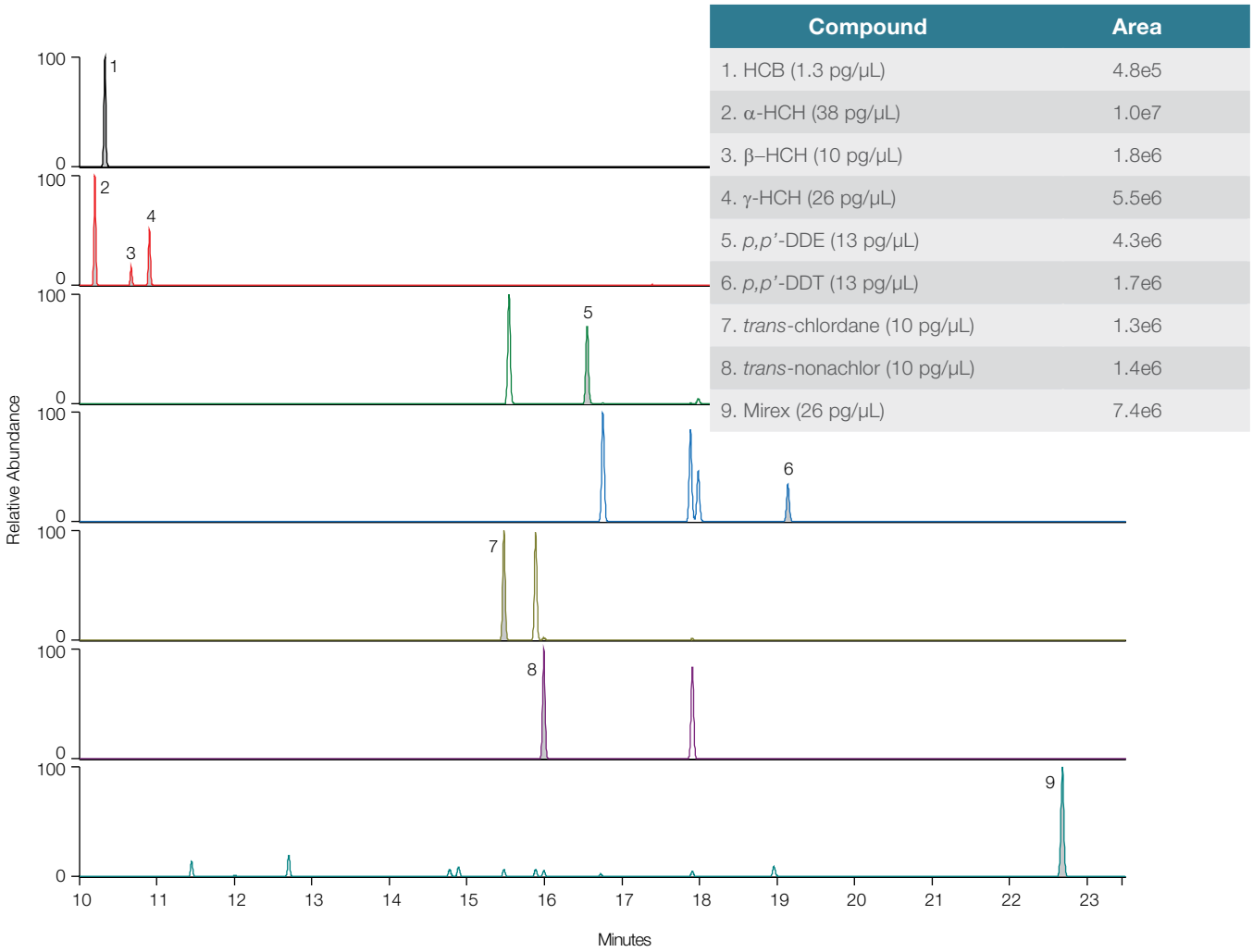
Full-scan acquisition with triple quadrupole sensitivity

Examples of the levels of sensitivity obtained for targeted POPs using the Q Exactive GC Orbitrap mass spectrometer are illustrated in Figures 1 and 2. Excellent response was observed in full-scan mode for both PCBs and OC pesticide quantification in solvent standards. Several compounds typically found within biological matrices within remote regions are highlighted, with area response ranging from 4e5 to 1e7 for injection of 1 to 38 pg on column (compound dependent). Substantial chemical noise can be observed in the full-scan analysis of the penguin blood extract (Figure 3). Total ion count (TIC) greater than 1e10 indicates substantial co-extracted sample matrix remains despite the clean-up strategies employed. However, even in the presence of such high levels of chemical background, detection of several PCBs (28/31, 66, 153) and OC pesticides (HCB, *pp*-DDE, and mirex) (Figure 4) could be achieved in full-scan acquisition. The high resolving power of the Q Exactive GC Orbitrap mass spectrometer provided selective mass separation (using ±5 ppm extraction window) of targeted compounds from co-extracted matrix ions.



Compound (7 pg/µL)	Area
1. PCB 28/31	3.8e6
2. PCB 52	1.2e6
3. PCB 101	1.0e6
4. PCB 118	1.2e6
5. PCB 138	7.5e5
6. PCB 153	8.9e5
7. PCB 180	6.0e5

Figure 1. Extracted ion chromatogram from full-scan acquisition of tri-, tetra-, penta-, hexa-, and heptachlorinated PCBs within a quantification standard (7 pg/µL)



Compound	Area
1. HCB (1.3 pg/µL)	4.8e5
2. α-HCH (38 pg/µL)	1.0e7
3. β–HCH (10 pg/µL)	1.8e6
4. γ-HCH (26 pg/µL)	5.5e6
5. *p,p'*-DDE (13 pg/µL)	4.3e6
6. *p,p'*-DDT (13 pg/µL)	1.7e6
7. *trans*-chlordane (10 pg/µL)	1.3e6
8. *trans*-nonachlor (10 pg/µL)	1.4e6
9. Mirex (26 pg/µL)	7.4e6

Figure 2. Extracted ion chromatogram from full-scan acquisition of OC pesticides quantification standard. Concentrations of individual pesticides range from 1 to 38 pg/µL.

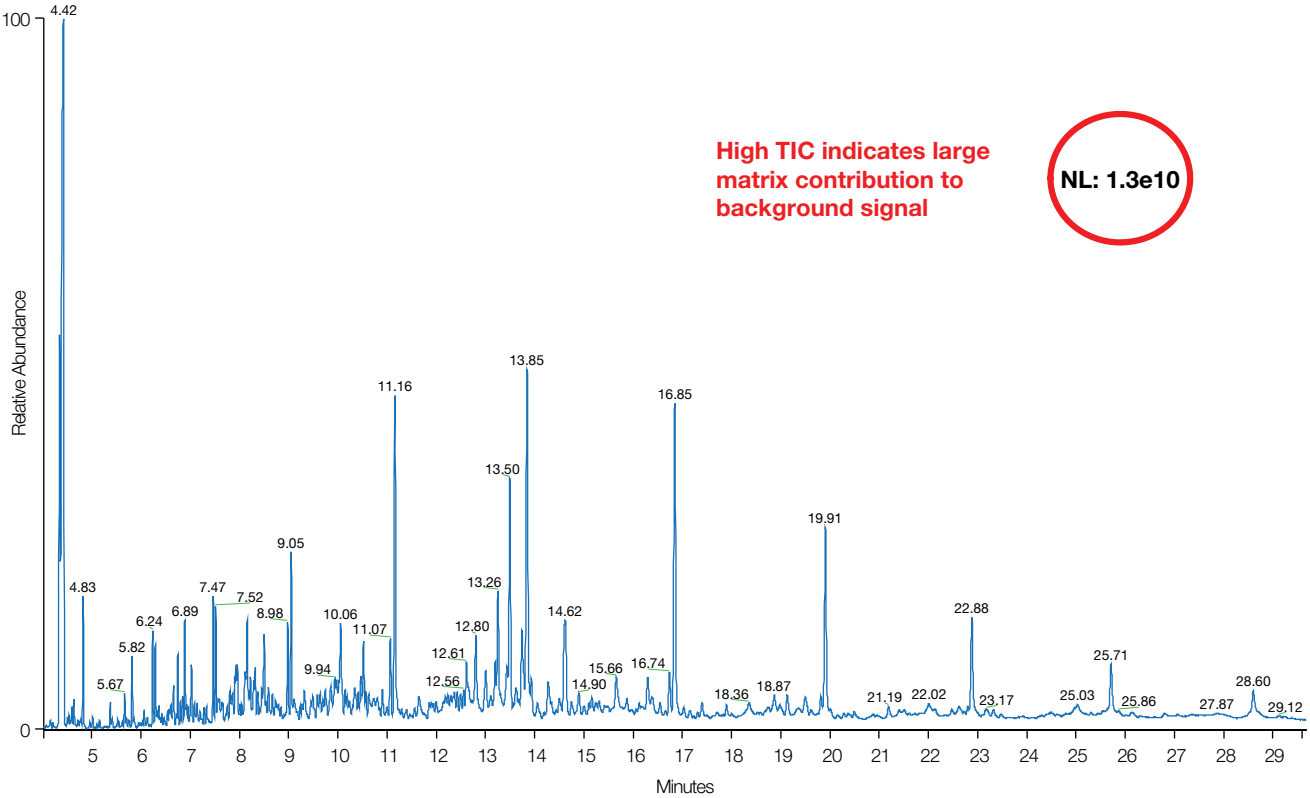


Figure 3. Full-scan total ion chromatogram (TIC) of King penguin blood extract highlighting substantial chemical noise from co-extracted sample matrix remains in final extract

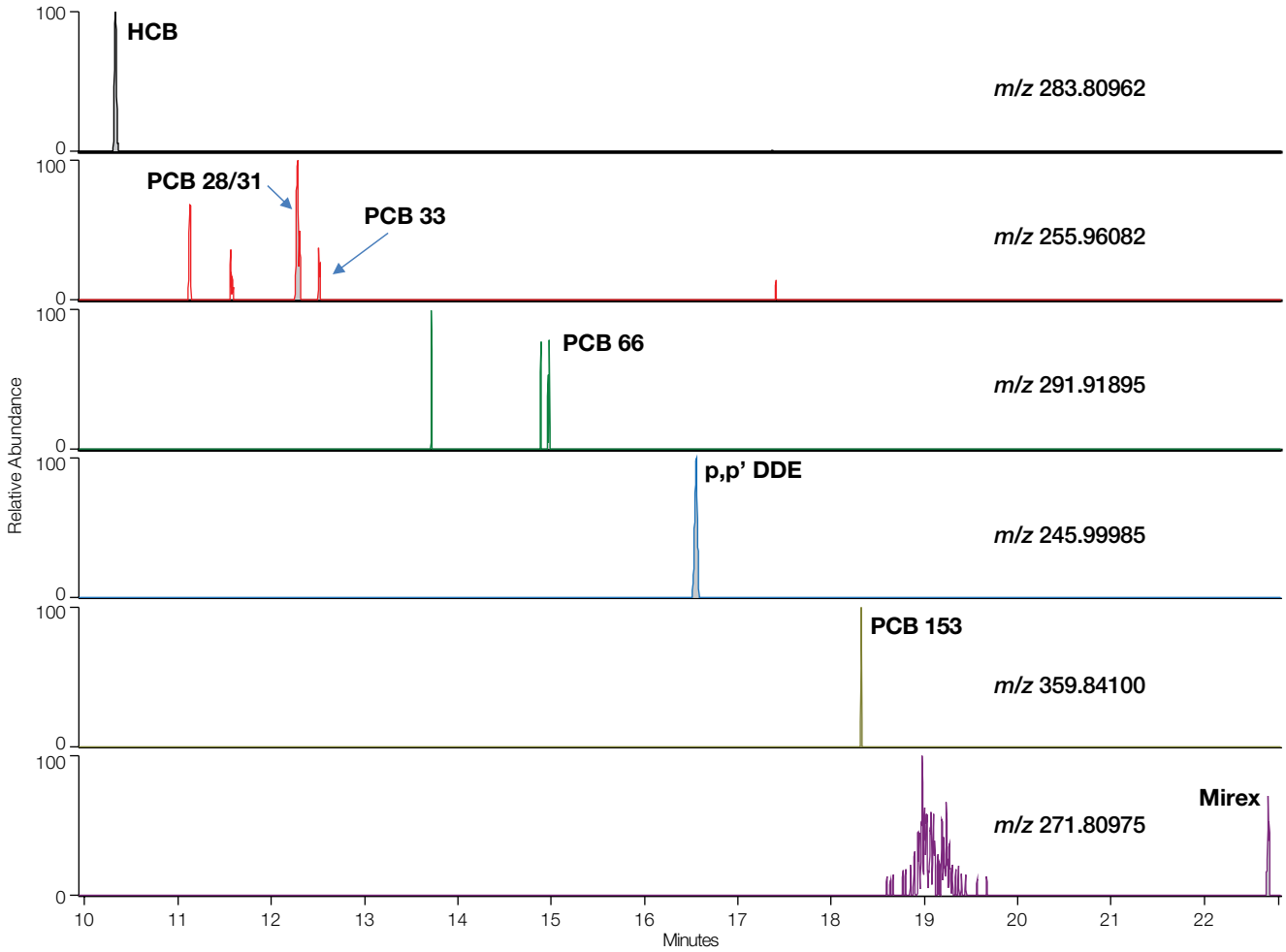


Figure 4. Extracted ion chromatogram of detected PCBs and OC pesticides in King penguin blood acquired in full-scan acquisition

Enhanced sensitivity/detection capabilities

Data acquisition using t-SIM mode can help improve detection limits/capabilities even further in complex samples in which significant co-extracted sample matrix remains. By utilizing the advanced quadrupole technology (AQT), mass regions of interest (i.e., isolation windows) can be targeted while minimizing introduction of co-extracted sample matrix into the C-trap of the Q Exactive GC Orbitrap system. Analysis using t-SIM acquisition of the same King penguin blood extract run previously in full-scan mode showed an improvement in the number of PCBs (Figure 5) and OC pesticides (Figure 6) detected. In addition to PCB 28/31, 33, 66, and 153 detected in full-scan (Figure 4), several other PCB congeners were detected using t-SIM acquisition (Figure 5). Similar findings were also observed for OC pesticides with the HCH isomers, *p,p'*-DDD, *p,p'*-DDT, chlordane, and nonachlor isomers being detected along with HCB, *p,p'*-DDE, and mirex (Figure 6).

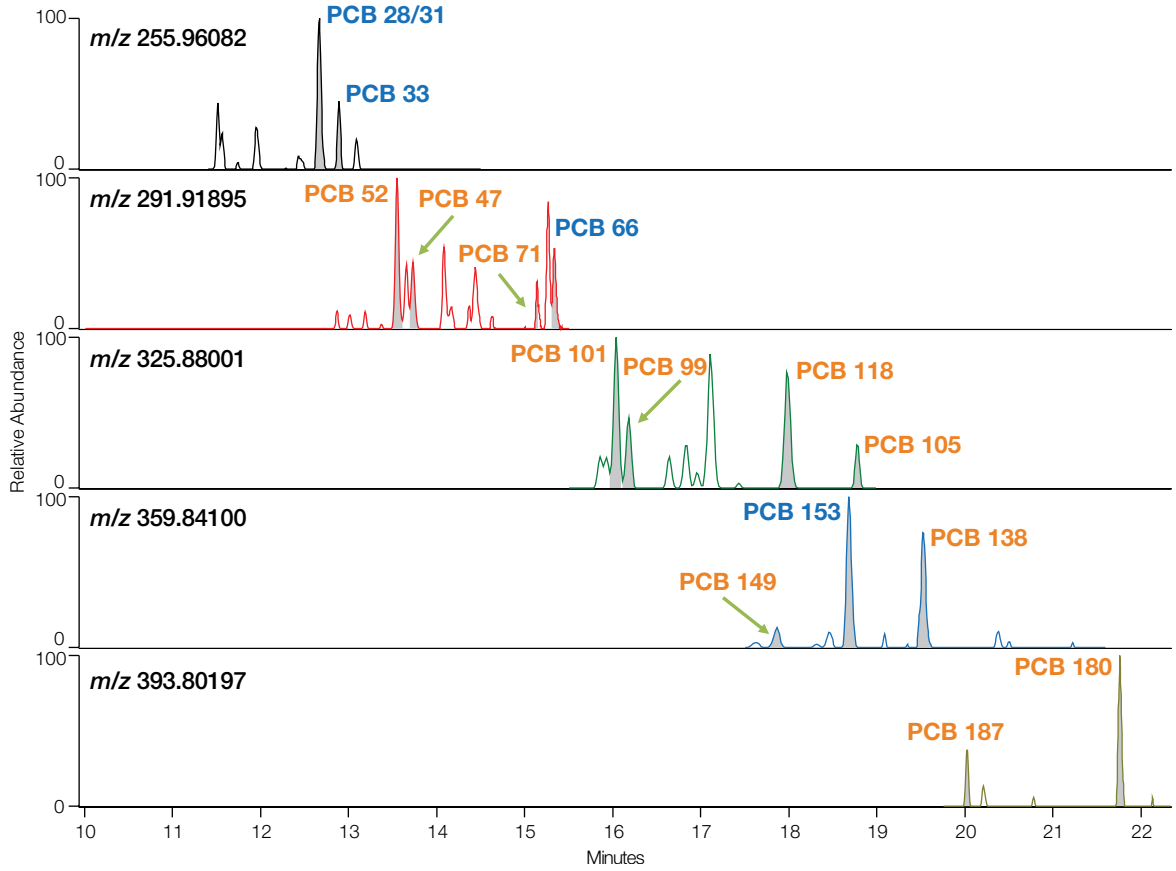


Figure 5. Extracted ion chromatogram of detected PCBs in King penguin blood extract with t-SIM acquisition. Compounds marked in green were not detected in full-scan acquisition.

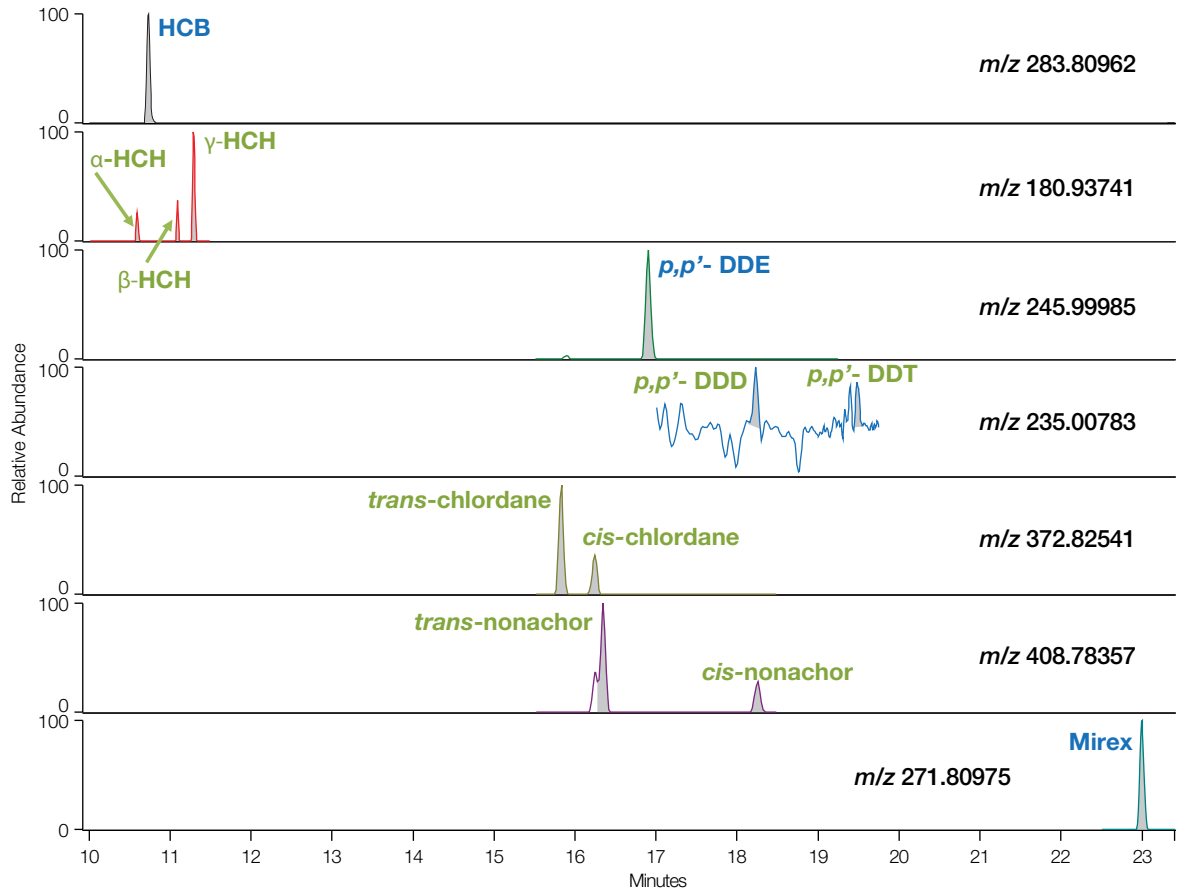


Figure 6. Extracted ion chromatogram of detected OC pesticides in King penguin blood from t-SIM acquisition. Compounds marked in green not detected in full-scan only acquisition.

Quantification of POPs in penguin samples

Detected compounds above blank levels were present at part per trillion (ppt) concentrations within King penguin blood (Tables 3 and 4). Highest concentrations were observed for HCB (290 pg/g ww), while *cis*-nonachlor was detected at 0.9 pg/g ww, demonstrating the sensitivity of the Q Exactive GC Orbitrap system. Limits of quantification (LOQ) were compound-dependent based on blank levels and ranged from 0.1 to 14.2 pg/g ww. Using t-SIM acquisition mode, the Q Exactive GC Orbitrap system was able to remove mass regions containing co-extracted sample matrix entering the C-trap, improving detection frequency at the low part per trillion concentrations.

Table 3. Concentration of PCBs (pg/g wet weight) present in King penguin blood sample

PCB Congener	Concentration (pg/g ww)
52	11.5
101	16.3
118	6.4
138	13.2
149	3.8
153	11.6
180	5.3
183	1.4
187	2.0

Table 4. Concentration of OC pesticides (pg/g wet weight) present in King penguin blood sample

Pesticide	Concentration (pg/g ww)
HCB	290
aHCH	3.9
p,p'-DDT	1.4
p,p'-DDD	4.7
p,p'-DDE	101
trans-chlordane	11.7
cis-chlordane	3.5
trans-nonachlor	11.7
cis-nonachlor	0.9
Mirex	38

Increased compound coverage using simultaneous full-scan/t-SIM acquisition

Comparison of results obtained between the full-scan and t-SIM acquisition show that co-extracted sample matrix entering the C-trap can hinder detection of targeted compounds at the low part per trillion-concentration range in full-scan acquisition. However, full-scan mass spectral data can still be obtained without sacrificing trace level sensitivity of compounds for targeted analysis. With the Q Exactive GC Orbitrap system, full-scan and t-SIM data acquisition can be obtained simultaneously within a single GC run. As t-SIM selectively isolates mass windows around compounds of interest, lower mass resolution settings can be utilized to insure an optimal number of data scans is obtained across chromatographic peaks while maintaining sufficient mass resolution for compound identification/detection. Assessment of sensitivity/detection frequency within King penguin blood using simultaneous full-scan (at 60,000 mass resolution)/t-SIM acquisition (30,000 mass resolution) was carried out. The number of PCB congeners detected in simultaneous full-scan/t-SIM acquisition (14) was slightly less that that obtained from t-SIM alone (16), but much greater when obtained in full-scan only acquisition (4) (Figure 7). Detection frequency decreased for OC pesticides as HCH isomers, *p,p'*-DDD, *p,p'*-DDT, and *cis*-chlordane were not detected in the simultaneous full-scan/t-SIM acquisition. However, detection frequency was still greater compared to that obtained from full-scan acquisition alone. This demonstrates that the Q Exactive GC Orbitrap system can still maintain trace level sensitivity while acquiring full-scan data simultaneously for identification of unknown compounds that may pose potential environmental/health risks.

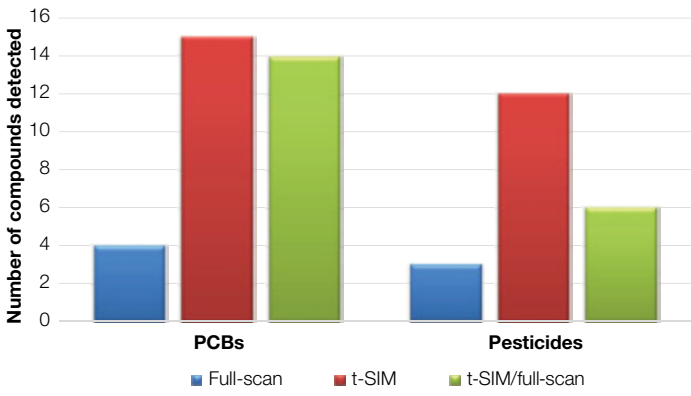


Figure 7. Number of PCBs and OC pesticides detected in King penguin blood in full-scan (blue), t-SIM (red), and simultaneous full-scan/t-SIM acquisition (green). Simultaneous full-scan/t-SIM acquisition utilized 30,000 resolution in for t-SIM acquisition and 60,000 resolution in full-scan acquisition.

Conclusion

The results of this study demonstrate that the Q Exactive GC Orbitrap high-resolution mass spectrometer provides excellent sensitivity, selectivity, and versatility in meeting the analytical challenges associated with trace level quantification of environmental samples from remote regions.

- Despite considerable co-extracted matrix interference, detection of several POP substances at part per trillion levels was achieved using full-scan acquisition at 60,000 mass resolution.
- Improvements in detection limits/capabilities could be obtained using t-SIM acquisition. The number of compounds detected in King penguin blood increased up to a factor of 4 using t-SIM.
- The processing power of the Q Exactive GC Orbitrap GC-MS/MS system provided the means of collecting full-scan mass spectra data without significantly compromising sensitivity of targeted analysis. Simultaneous full-scan and t-SIM data acquisition provided quantification of targeted analytes at part per trillion levels while collecting full-scan mass spectral data for identification of potential unknown compounds impacting remote environments.

Acknowledgments

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Discovery of Emerging Disinfection By-Products in Water Using Gas Chromatography Coupled with Orbitrap-based Mass Spectrometry

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Application Note 10490

Key Words
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.

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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 below (Tables 1 and 2).

Table 1. GC Temperature program.

TRACE 1310 GC 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 below for untreated-control and chlorinated samples.

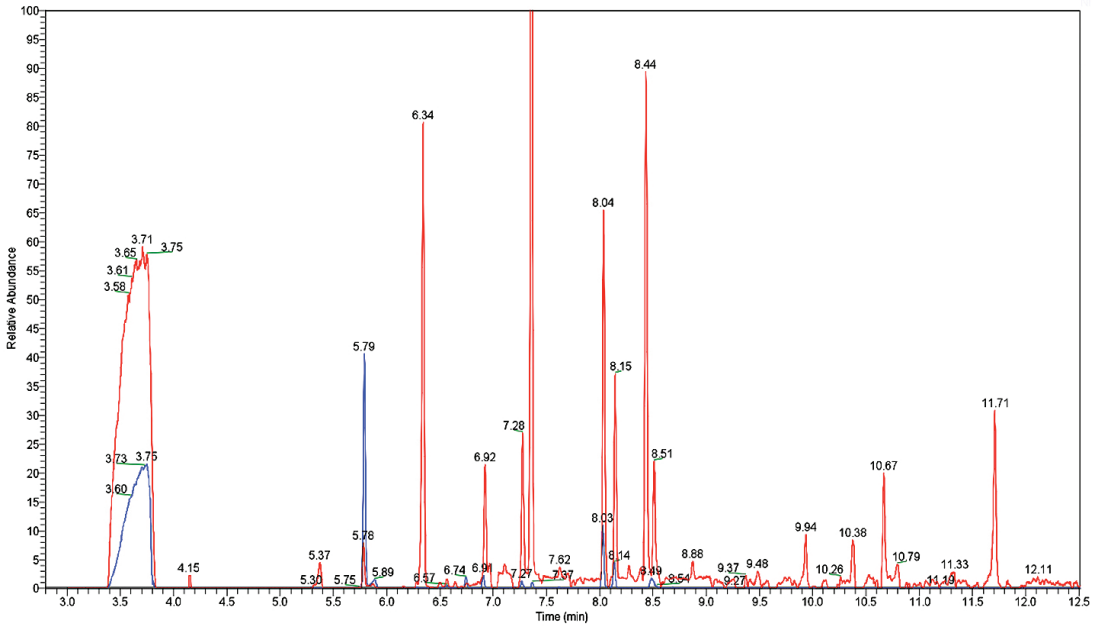


Figure 1. Overlayed extracted ion chromatograms (*m/z* 126.90392, iodine) of Milli-Q water spiked with natural organic matter (NL NOM) subjected to chlorination (red) and control of untreated water (blue) showing an increase in both the number and intensity of iodine-containing peaks in the chlorinated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

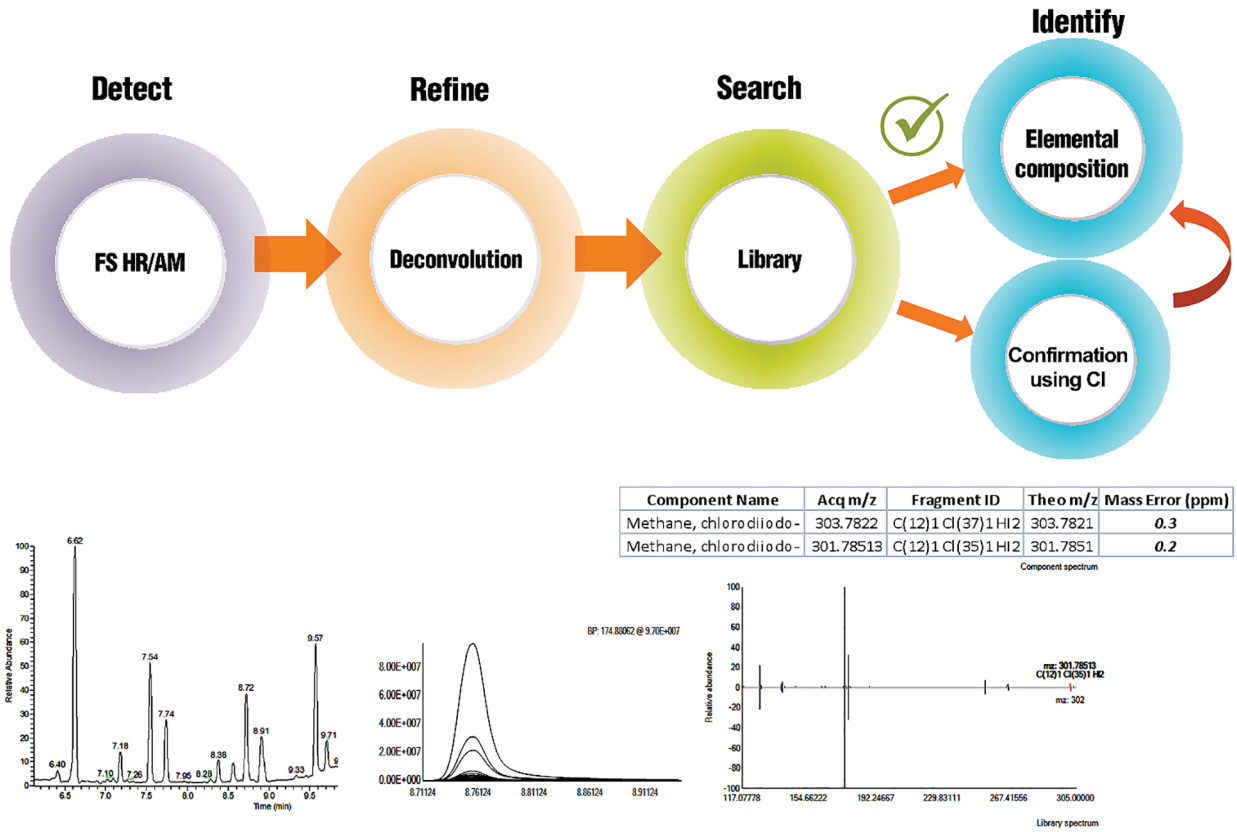


Figure 2. Compound discovery workflow used for iodo-DBPs peak detection with spectral deconvolution and tentative compound identification.

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.

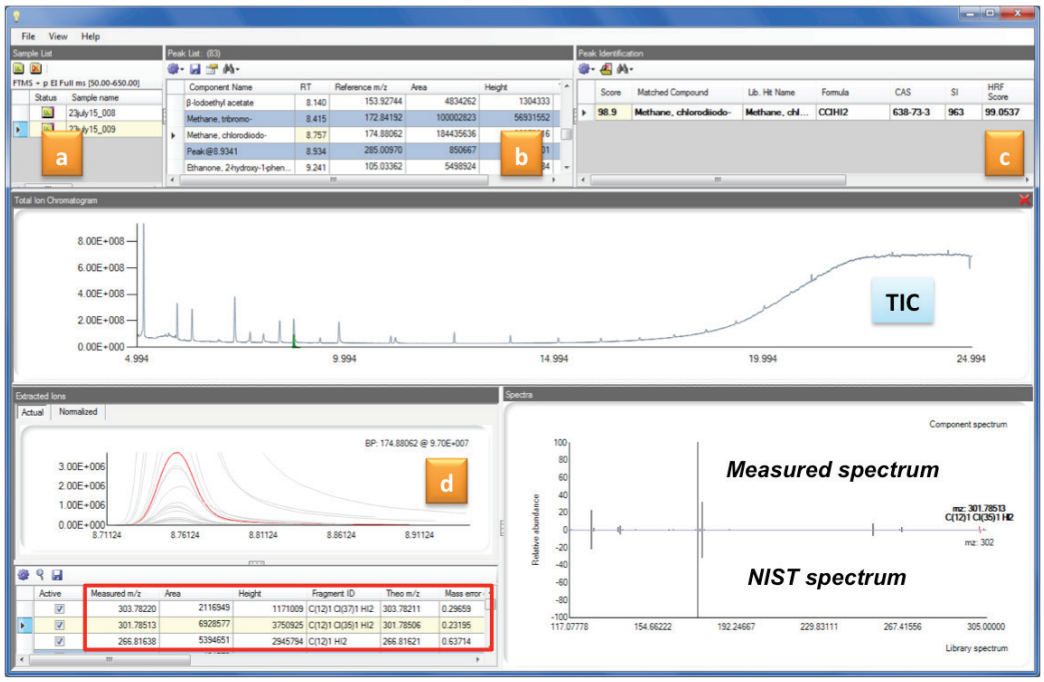


Figure 3. Deconvolution browser showing chlorodiiodomethane identification based on library (NIST) match search index, SI 963), fragment rationalization with an HRF› 99% and mass accuracies of measured fragments (e.g., molecular ion *m/z* 301.78513 ppm = 0.23). Samples processed (a), peaks detected (b),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

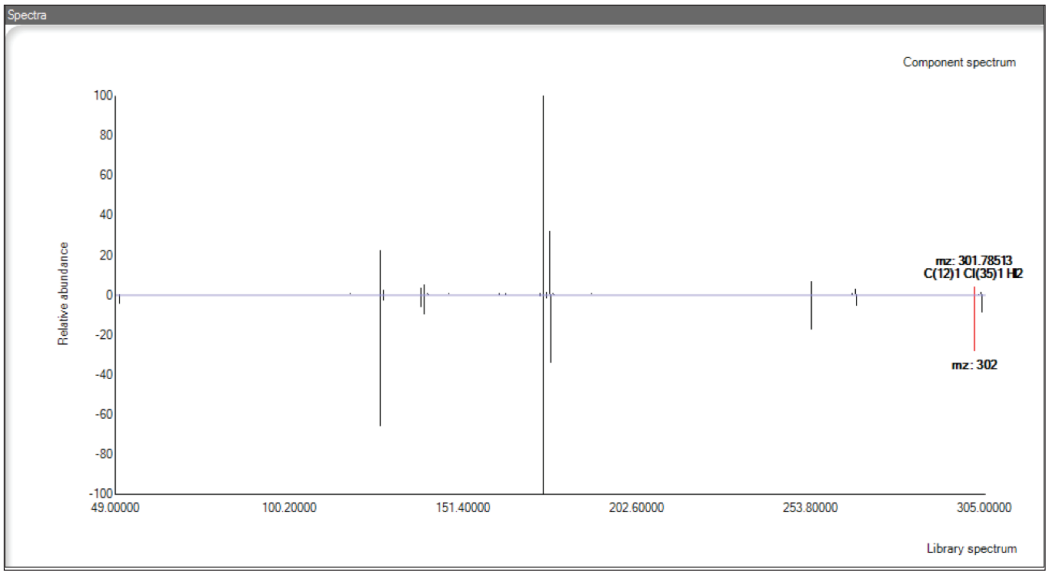


Figure 4. Ion mass spectrum, corresponding accurate masses (ppm) and elemental composition of chlorodiiodomethane (RT= 8.77 min) a) in the chloraminated NL NOM extract and b) MS library match. Data acquired in EI at 60,000 resolution (FWHM, at *m/z* 200). Annotated are the acquired fragment ions that 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).

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.

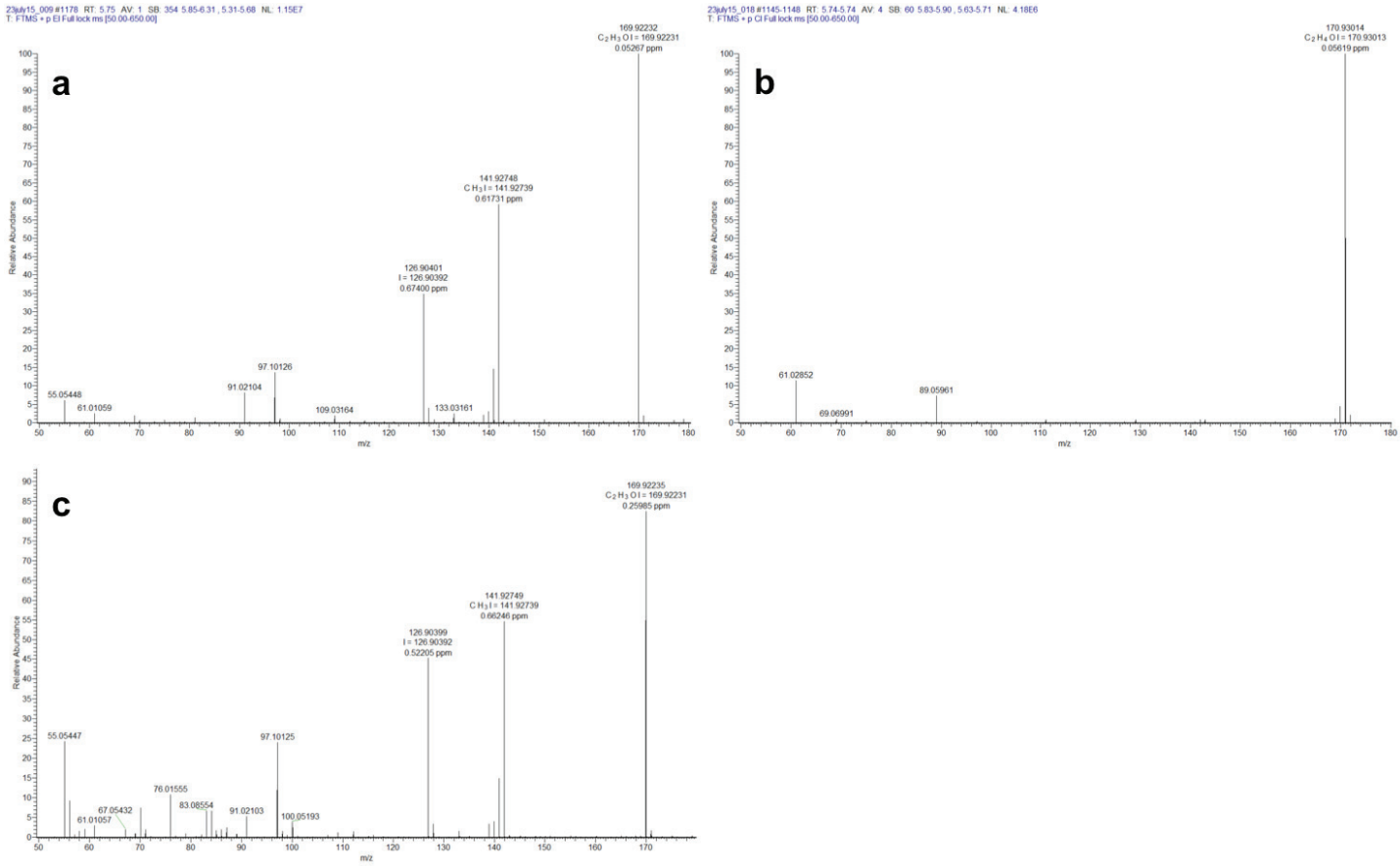


Figure 5. Confirmation of iodoacetaldehyde identification with authentic solvent standard (a) and NL treated samples (c) based on RT and mass accuracy measurements. Positive chemical ionization (PCI) mass spectrum (b) confirms mass of molecular ion $[M+H]^+$ with 0.06 ppm mass accuracy.

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.

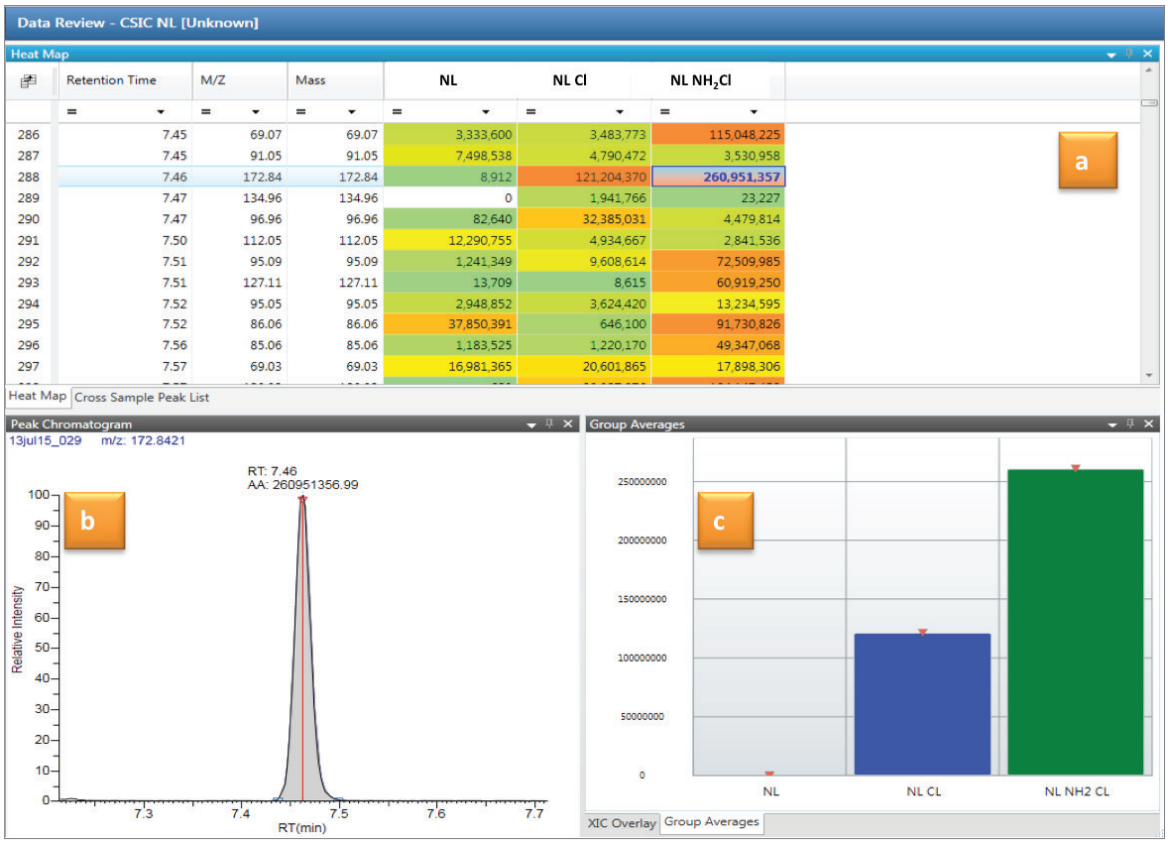
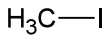
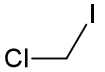
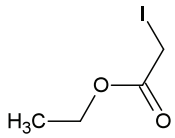
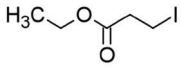


Figure 6. TraceFinder browser showing the heat map with the peak areas of detected peaks (a), and as an example, the increased concentration of a compound eluting at RT = 7.46 min, the corresponding extracted peak chromatogram (b), and the abundance of this chemical in the samples analyzed (c).

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).

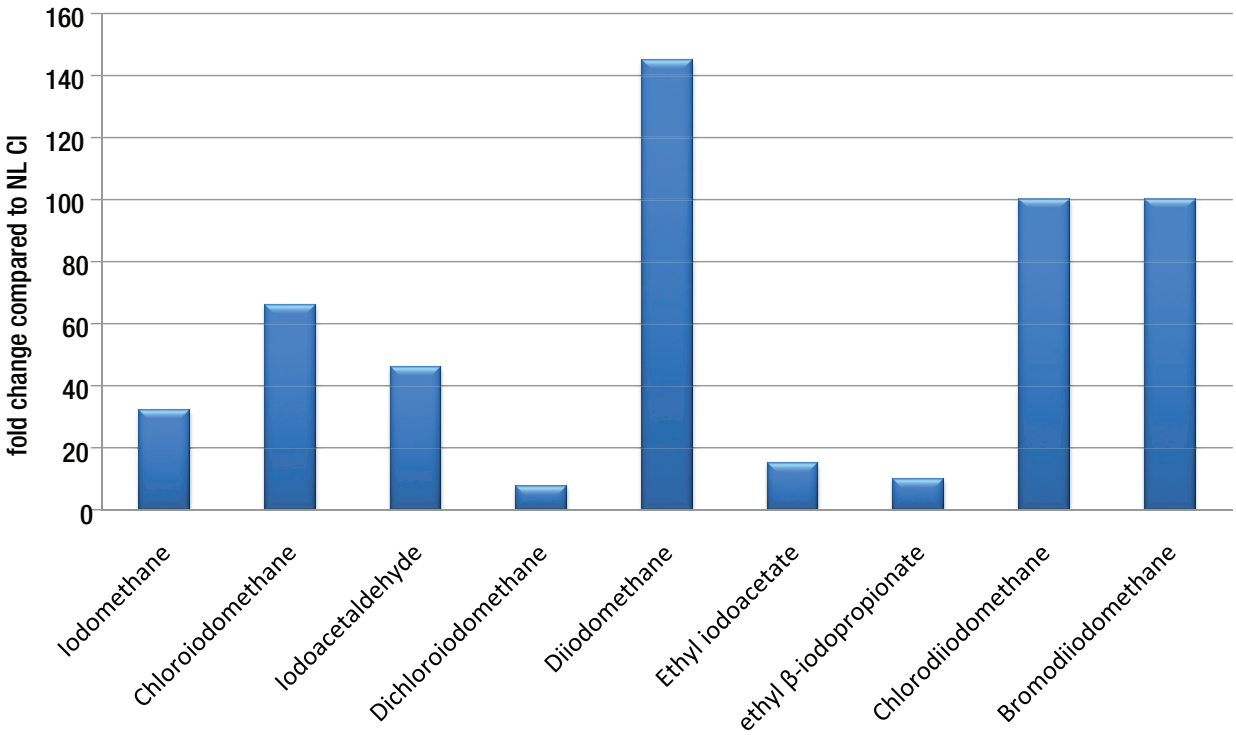


Figure 7. Fold increase of iodo-DBPs detected and identified in chloraminated DBP mixture concentrates as compared to chlorinated ones.

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.

Acknowledgements

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References

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3. Postigo C., Cojocariu CI., Richardson SD., Silcock P., Barcelo D. Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters using Orbitrap-based gas chromatography-mass spectrometry, *Analytical and Bioanalytical Chemistry,* DOI 10.1007/s00216-016-9435-x.
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TECHNICAL NOTE 10511

Full-scan Analytical Performance

Thermo Scientific Exactive GC and the Thermo Scientific Q Exactive GC Mass Spectrometers

Cristian Cojocariu[1], Dominic Roberts[1] and Paul Silcock[1] [1] Thermo Fisher Scientific, Runcorn, UK

Goal

The Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS System and the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS System have been designed to provide equivalent performance when using full-scan acquisition modes. The objective of this study was to test the analytical performance of the Exactive GC system and the Q Exactive GC system using full-scan acquisition. Both mass spectrometers were evaluated for key analytical parameters such as scan speed, sensitivity, mass accuracy, dynamic range and linearity.

Experimental

In all experiments, a Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer and a Thermo Scientific Exactive GC mass spectrometer were used. Sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation of the analytes of interest was obtained using a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and a Thermo Scientific™ TraceGOLD™ TG-5SilMS, 30 m × 0.25 mm × 0.25 µm film capillary column (P/N: 26096-1425). The Exactive GC system and the Q Exactive GC system were tuned and calibrated using PFTBA over exactly the same mass range to achieve mass accuracy of <1.0 ppm. The default ionization mode was electron ionization (EI) and the mass spectrometers were operated using full-scan 60,000 resolution (FWHM, measured at *m/z* 200) (Table 1). Data acquired was lock-mass corrected using siloxane masses from the GC column bleed. (Table 1)

Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	full-scan
Mass range (Da):	50-450
Mass resolution (FWHM at *m/z* 200):	60k
Lockmass, column bleed *(m/z)*:	207.03235 281.05114 355.06993

TRACE 1310 GC System Parameters	
Injection Volume (µl):	1.0
Liner	LinerGOLD™, single taper (P/N:453A0344-UI)
Inlet (°C):	250
Inlet Module and Mode:	splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold Time (min):	1.0
Temperature 2 (°C):	250
Rate (°C/min)	30
Hold Time (min):	0.0
Temperature 3 (°C):	150
Rate (°C/min)	30
Temperature 3 (°C):	320
Rate (°C/min)	2.0

Table 1. Gas chromatography and mass spectrometers analytical parameters.

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Scan speed

The Exactive GC system and the Q Exactive GC system provide the same scan rates when set to the same resolving power. An example is shown in Figure 1, where the same number of data points across a three second wide peak (extracted ion chromatogram of *m/z* 179.17923) was obtained. Additional information about the Q Exactive GC system was published before.[1, 2]

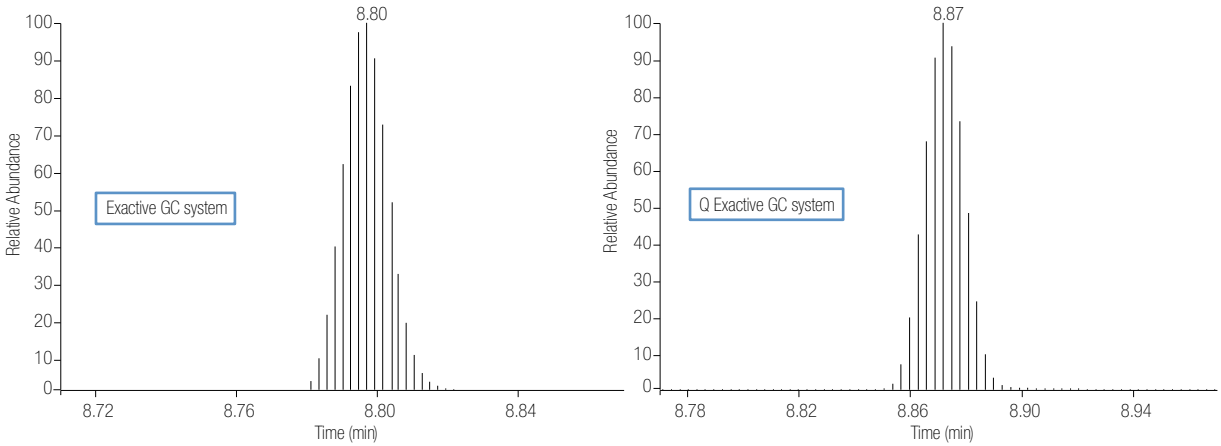

Figure 1. Comparable scan speed demonstrated for 2-butyl-5-hexyloctahydro-1H-Indene measured on the Exactive GC and the Q Exactive GC systems. Both mass spectrometer systems operated in full-scan at 60k resolution (FWHM at *m/z* 200).

Sensitivity

To test if the two mass spectrometers have similar sensitivity, an 8270 semivolatile MegaMix® working solution (Restek) in dichloromethane was diluted to 1 pg/µL (in dichloromethane) and analyzed simultaneously using the Exactive GC and the Q Exactive GC systems. Replicate injections (n=8) of this standard were performed on two different days. The instrument detection limits (IDL) of selected chemicals in the mixture were calculated for each mass spectrometer on both days and the average values are reported in Figure 2. The IDL was calculated taking into account the Student's-*t* critical values for the corresponding degrees of freedom (99% confidence).[2] Both systems demonstrated excellent sensitivity with IDL values ranging from 0.04 to 0.17 pg/µl. The results of this experiment confirmed that the Exactive GC system and the Q Exactive GC system are able to detect comparable levels of analytes.

Compound	RT (min)	Exactive GC System	Q Exactive GC System
Nitrobenzene	5.53	0.11	0.10
Isoforone	5.75	0.06	0.10
4-Nitroaniline	7.44	0.06	0.10
Acenaphthene	7.93	0.05	0.07
Diphenylamine	8.55	0.04	0.09
Hexachlorobenzene	9.01	0.06	0.13
Phenanthrene	9.40	0.15	0.15
Carbazole	9.61	0.07	0.08

Figure 2. Instrument detection limits (IDLs, as pg on-column) calculated for selected analytes. Data represents repeat injections (n=8) of a 1.0 pg on-column 8270 semivolatile solvent standard. Two sets of measurements were acquired in two distinct days using the Exactive GC and the Q Exactive GC systems. Error bars annotated represent one standard deviation calculated from the two sets of measurements. Standard deviation calculated from the two sets of measurements is annotated.

Linear dynamic range

A wide linear dynamic range over which accurate mass measurements are to be made is essential, especially when dealing with applications where the samples analyzed contain a complex and varied chemical background that could potentially interfere with the analytes of interest (ex: routine pesticide screening, metabolomics). To test if the two mass spectrometers have similar linear dynamic ranges, repeat injections (n=3) of increasing concentration levels (0.1 pg to 10,000 pg on-column) of the 8270 semivolatile mix were performed. An example of compound linearity obtained from both mass spectrometers is shown in Figure 3 for hexachloroethane, the results demonstrating equivalent performance.

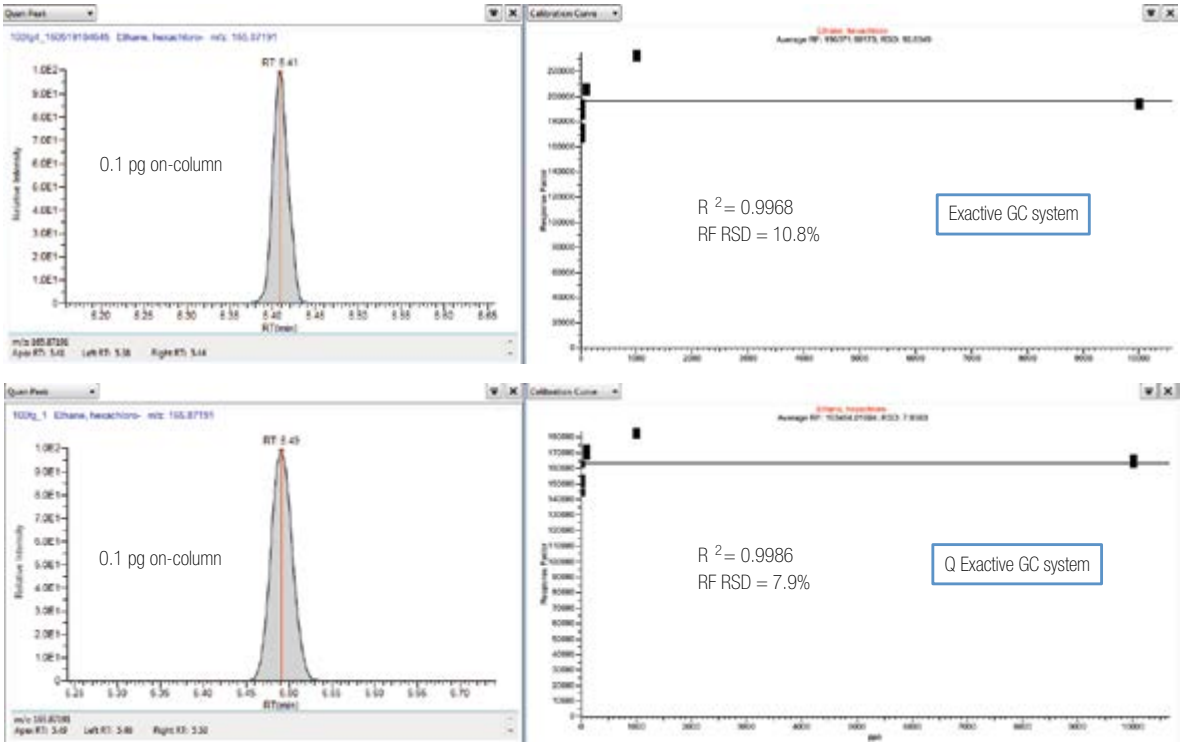

Figure 3. Linear dynamic range of the Exactive GC system and the Q Exactive GC system demonstrated using hexachloroethane solvent standards injected over six orders of magnitude. Extracted ion chromatogram (*m/z* 165.87191) corresponding to hexachloroethane at 0.1 pg on-column is shown together with the coefficient of determination (R^2) and the RF %RSD values determined over a concentration range of 0.1-10,000 pg on-column.

Moreover, excellent peak area repeatability (n=3 injections) was obtained on both mass spectrometers at each concentration level as demonstrated for hexachloroethane in Table 2 which shows %RSD ranged from 0.6-6.9% across the six orders of magnitude.

Hexachloroethane concentration (pg on-column)	Exactive GC system %CV (n=3)	Q Exactive GC system %CV (n=3)
10000	0.8	1.7
1000	0.6	0.6
100	0.9	1.6
10	1.4	1.2
1	0.8	2.1
0.1	6.9	3.6

Table 2. Calculated % CV from n=3 repeat injections of hexachloroethane solvent standard at various on-column concentrations. Data from the Exactive GC system and the Q Exactive GC system is shown.

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Maintaining sub-ppm mass accuracy irrespective of compound concentration

Sub-ppm mass accuracy was maintained across compound concentrations on both mass spectrometers as exemplified below for hexachloroethane. In all cases, irrespective of the *m/z* and concentration level, <1ppm mass accuracy was obtained on both the Exactive GC and the Q Exactive GC systems. This is essential as any compromise in accuracy of mass measurements can result in false identification and non detection of compounds of concern, such as pesticides in a screening experiment.[1]

Level	EGC	QEGC	EGC	QEGC	EGC	QEGC	EGC	QEGC	EGC	QEGC
ppb on-column	*m/z* 118.90306	*m/z* 118.90306	*m/z* 116.90601	*m/z* 116.90601	*m/z* 120.90011	*m/z* 120.90011	*m/z* 165.87191	*m/z* 165.87191	*m/z* 202.83781	*m/z* 202.83781
0.1	0.6	0.1	0.8	0.1	0.5	0.2	0.3	0.2	0.7	0.1
1	0.7	0.1	0.5	0.2	0.4	0.1	0.1	0.7	0.4	0.8
10	0.4	0.1	0.3	0.0	0.3	0.1	0.4	0.7	0.5	0.9
100	0.2	0.1	0.1	0.1	0.3	0.2	0.8	0.7	1.0	1.0
1000	0.8	0.4	0.5	0.2	0.4	0.1	0.7	0.4	0.9	0.7
10000	0.5	0.4	0.5	0.3	0.5	0.2	0.6	0.3	1.0	0.7
average Δppm	**0.5**	**0.2**	**0.4**	**0.2**	**0.4**	**0.1**	**0.5**	**0.5**	**0.7**	**0.7**

Figure 4. Comparative mass accuracy (Δ ppm) measurements for several hexachloroethane ions over > 5 orders of magnitude using the Exactive GC (EGC) and the Q Exactive GC (QEGC) systems. Average mass accuracy (Δppm) value for each ion is also indicated.

Conclusions

Overall, the experimental data shown here demonstrate that the Exactive GC and the Q Exactive GC mass spectrometers deliver comparable high-quality analytical performance using full-scan acquisiton.

Both systems have the same fast scan speed, allowing the analyst to obtain sufficient data points across narrow chromatographic peaks, to accurately describe the peak area and to ensure signal reproducibility.

The sensitivity of the Exactive GC system and the Q Exactive GC system used for this study is also very similar and this was demonstrated from the IDL values obtained for the test compounds.

The linear dynamic range was also comparable on both systems, extending to six orders of magnitude (0.1-10,000 pg on-column) as demonstrated for selected compounds.

Mass accuracy was consistently maintained on both mass spectrometers at sub-ppm levels irrespective of compound concentration.

References

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2. Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology. Application Note 10449. [Online] : https://tools.thermofisher.com/content/sfs/brochures/AN-10449-GC-MS-Orbitrap-Pesticides-Baby-Food-AN10449-EN.pdf

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CASE STUDY
Agenzia Provinciale per l'ambiente
(Provincial Agency for the Environment)

GC Orbitrap MS/MS system addresses an expanding set of compounds of concern to health and the environment

"Our analyses must cover more than 50,000 compounds, so we need an instrument that can be used for many purposes. Our choice was the Q Exactive GC Orbitrap system."

—Dr. Flavio Ciesa, Safety chemicals (REACH) and chromatographic analysis, Agenzia Provinciale per l'ambiente

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Photo courtesy of the Provincial Agency for the Environment

"If we find something that doesn't look correct with a sample, or if we want to check a sample later for other contaminants, with the Q Exactive GC Orbitrap GC-MS/MS system we can go back to the full-scan data to determine with good sensitivity what is there, which is impossible with a single quadrupole system in SIM mode or a triple quadrupole in SRM mode."

—Dr. Flavio Ciesa

Protecting human health and the environment against the risks of chemicals

Human health is not only affected by contaminants in foods and the environment, it also influenced by the products—clothes, toys, cosmetics, and detergents—used daily. These are the concerns of the Agenzia Provinciale per l'ambiente (the Provincial Agency for the Environment) of Bolzano, Italy. In collaboration with private and public groups, its laboratories perform chemical and biological analyses of foods, beverages, feed, textiles, toys, cosmetics, detergents, jewelry, and more.

Using the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system, the Agency is able to analyze a large and expanding list of compounds of interest to human health and the environment, with greater certainty and ease.

Food Analysis Laboratory interests extend beyond food and environmental samples

- Determination of phthalates (plasticizers) in toys
- Control of aromatic amines and carcinogenic dyes in textile products
- Testing for carcinogenic chemicals such as aromatic amines in inks used for tattoos and permanent makeup

European Union regulations increase the scope of compounds monitored

Recent European Union (EU) chemical safety, REACH, and CLP regulation is driving need for methods able to analyze a large and growing number of substances of interest. REACH (Registration, Evaluation, Authorization of CHemicals) aims to improve the protection of human health and the environment against the risks of chemicals, increase the competitiveness of the European chemical industry, and reduce the number of tests carried out on animals by promoting alternative methods for assessing substances. CLP (Classification, Labeling and Packaging) regulates the classification, labeling and packaging of substances and mixtures.

"When we have complex sample matrices, the Q Exactive GC Orbitrap GC-MS/MS system makes it a lot easier because we can prepare samples using a general method."

—Dr. Flavio Ciesa

The Q Exactive Orbitrap GC-MS/MS system addresses expanding diversity of targeted and non-targeted analyses

With a broad analytical charter expected to continue to expand due to emerging health concerns and regulations, the laboratory needed an instrument that can be used to confidently detect and quantify a wide variety compounds in complex sample matrices. When unknown peaks are found, the laboratory often performs untargeted analyses to determine their identities.

With full-scan high-resolution accurate-mass (HRAM) capability, and the ability to perform MS/MS experiments and check isotopic abundance patterns, the Q Exactive GC Orbitrap GC-MS/MS system delivers more than high-capacity high-confidence screening and quantification. Using the Q Exactive GC Orbitrap GC-MS/MS system, the laboratory can also perform retrospective data analysis to identify compounds not detected using traditional targeted analyses, and to go back through data months or even years later to check for substances not previously targeted.

According to Dr. Flavio Ciesa of the REACH Analysis Laboratory, "When you work in full-scan you have a complete set of data that you can go back to. That's been a good thing to have because, for example, after analyzing some fish samples for PCBs we were able to go back to the data without rerunning samples and find tetra-, penta-, and hexachlorobenzenes."

High-resolution reduces interferences

Complex sample matrices present significant challenges to selectivity and detection limits, demanding that laboratories spend more time on sample preparation. With HRAM capability, the Q Exactive GC Orbitrap GC-MS/MS system provides excellent sensitivity, quantitative accuracy, precision, and linearity, even when a general method is used to prepare complex sample matrices.

Polychlorinated biphenyls (PCBs) are among the most widespread persistent organic pollutants and are identified in every compartment of the global ecosystem. Chromatographic separation is complex and no single GC-column can completely separate all of the 209 possible congeners. Interferences such as co-elution of higher homologues can make MS detection difficult. The high-resolution capability of the Q Exactive GC Orbitrap GC-MS/MS system is powerful in minimizing interferences when analyzing complex matrices for PCB contamination (Figure 1).

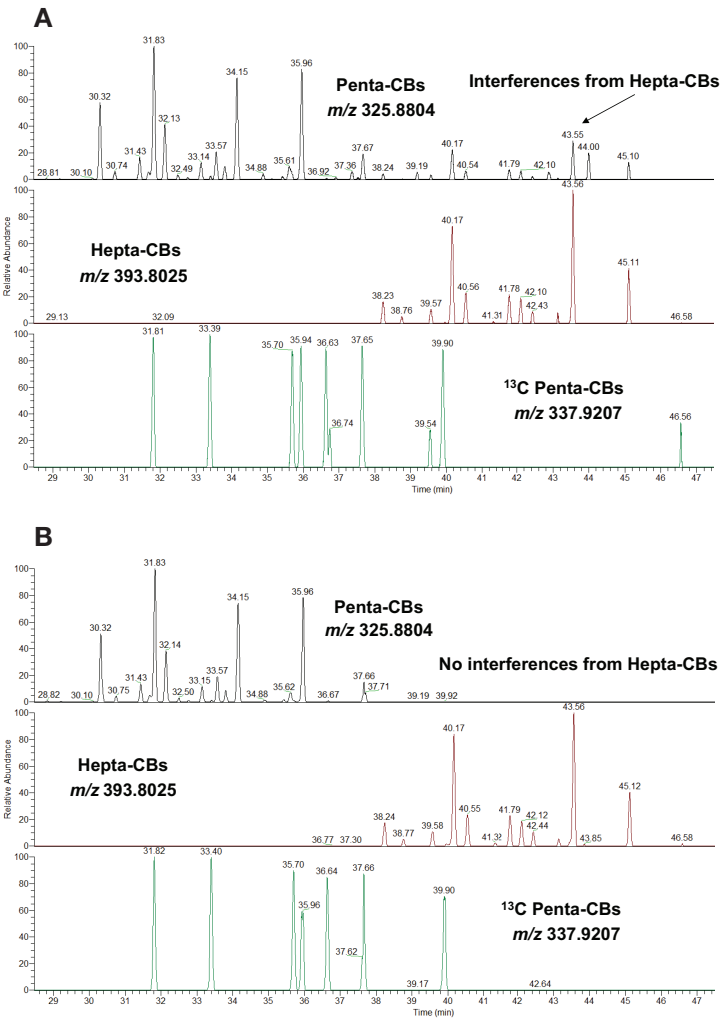

Figure 1. Resolving interferences when analyzing PCBs using the Q Exactive GC Orbitrap GC-MS/MS system. A. An ion chromatogram of a fish sample at 10,000 mass resolution shows interferences from hepta-chlorinated biphenyls (hepta-CBs). B. At 60,000 mass resolution, the hepta-CB interferences are eliminated. Courtesy of Ciesa, F. and D'Ambrosio, L. of the Laboratorio analisi alimenti; and Basso, A., Mair, K., Fellin, V., and Tirler, W. of Eco-Research, Bolzano, Italy.

Photo courtesy of the Provincial Agency for the Environment

"For multi-component applications I prefer Thermo Scientific™ TraceFinder™ software because the software is intuitive and time-saving and because it automatically performs complex calculations such as ion ratios."

—Dr. Flavio Ciesa

HRAM data ensure high-confidence results that withstand challenge

Since a product could be recalled if a contaminant is detected above safe levels, laboratory results must be of highest quality and certainty, able to withstand any scrutiny. As Dr. Ciesa explains, "The results of our experiments are very important, so we have to be sure the data we obtain are correct. Whether we have a standard to use or not, when we work at ≥ 60K resolution and obtain an exact mass, we can perform MS/MS experiments and can control the isotopic abundance pattern so the results are the best any lab could provide."

Conclusion

The Q Exactive GC Orbitrap GC-MS/MS system brings together the power of GC and HRAM Orbitrap MS to provide high-capacity targeted and untargeted component detection, even in extremely complex samples. Full-scan data acquisition makes it simple to perform targeted and non-targeted methods, and allows for a potentially unlimited number of compounds to be monitored a single sample injection. Unlike SRM acquisition on a triple quadrupole instrument, high-resolution, full-scan data acquisition enables retrospective interrogation of data to search for emerging contaminants that were not screened for when the data were originally acquired.

About Flavio Ciesa

Flavio Ciesa studied chemistry at the University of Parma, and obtained his Ph.D. in Innovative Material Science. In 2008, Dr. Ciesa joined the Laimburg Research Centre as a postdoctoral researcher where he was involved in untargeted metabolomics analysis using different techniques. From 2010 to 2014, he headed various different projects covering analyses of apples and wines. In 2014, he joined the provincial environment agency in Bolzano, where he currently works to address the safety of toys, textile, tattoo inks, chemicals, and other areas of importance to public health.

Provincial Palace in Via Amba Alagi 5, Bolzano.
Photo courtesy of the Provincial Agency for the Environment.

About the Agenzia Provinciale per l'ambiente

http://ambiente.provincia.bz.it/appa-bolzano.asp

The Agenzia Provinciale per l'ambiente (Provincial Agency for the Environment) is the largest South Tyrolean institution of experts in the technical protection of the environment, climate, and resources. "We work for the respectful use of natural resources and for their long-term preservation on the provincial territory," explains Flavio Ruffini, director of the provincial Environmental Agency, "in order to promote, today and in the future, a sustainable development of the territory and a better quality of life for its population." To achieve these goals, the Agency provides advice to municipalities and institutions, follows the requests of citizens, finances measures and initiatives, and develops measures for protection, prevention, verification and control. For the Agency, the protection of the environment and climate and environmental sustainability are also cultural tasks, for which it is active, sensitizing and informing, in the areas of sustainable development, food safety and climate protection.

Find out more at **thermofisher.com/OrbitrapGCMS**

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