New opportunities for the non target analysis of environmental contaminants gas chromatography Orbitrap mass spectrometry

Paul Silcock(1), Cristian I. Cojocariu(1), Jason Cole(2), Flavio Bedini(3) Fausto Pigozzo(3) (1)Thermo Fisher Scientific, Runcorn, UK. (2) Thermo Fisher Scientific, Austin, TX. (3)Thermo Fisher Scientific, Rodano, Italy.

ABSTRACT

Since the middle of the 20th century, Gas Chromatography Mass Spectrometry (GC-MS) has made a long journey towards its current status as one of the major analytical techniques in a diverse range of applications. Despite this, GC-MS has had more than four decades to wait for a new type of mass analyzer with the potential to advance capability over previously applied technology. Almost two years on from the first commercial introduction of Thermo Scientific™ Orbitrap™ GC-MS in 2015, in this poster, we explore how this technology has been applied specifically to the analysis of environmental contaminants and how we can use a highly selective non-target acquisition to explore changes in our approach to routine environmental analysis. Primary applications to be highlighted are the discovery of new disinfection by-products (DBPs) resulting from water treatment processes, using a non targeted approach; also, the potential for addressing the difficult analytical challenges for a complex class of emerging persistent organic pollutants, namely short chain chlorinated paraffins (SCCPs).

INTRODUCTION

Iodinated disinfection byproducts (DBPs) are considered an emerging class of DBPs of concern due to their potential toxic effects for human health. Some of the identified iodinated DBPs have been found to be more genotoxic and cytotoxic than the corresponding brominated and chlorinated analogues[1,2]. However, most iodo-DBPs still belong to the unknown fraction of the halogenated material (~50% of the TOX) formed during water chlorination and hence, remain unidentified[1]. In this context, the main goal of the work was to investigate the formation of iodinated DBPs during chlorination and chloramination of source waters with different iodide and bromide levels. Short Chained Chlorinated Paraffins (SCCPs) are emerging contaminants intentionally manufactured and can have harmful and irreversible effects for humans and the environment. SCCPs are listed in the Stockholm convention and their production and use in Europe is restricted and regulated. Detection and quantification of SCCPs is challenging because SCCPs are present in the environment at low levels and as very complex isomeric mixtures, difficult to separate chromatographically. 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. The experiments focused on assessing the sensitivity, linear dynamic range, selectivity and analytical precision for the analysis of SCCPs technical mixtures. Both electron ionization and negative chemical ionization were used.

MATERIALS AND METHODS

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 characteristics and 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 with 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 Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system for analysis of iodo-DBPs. Details about the procedures followed to perform the disinfection reactions and DBP analysis can be found elsewhere[3].

To test the performance of the GC-Orbitrap platform for the analysis of SCCP, two SCCP technical mixtures (63% and 55.5% chlorine) were used to test the performance of the Exactive GC system using both electron ionisation (EI) and negative chemical ionisation (NCI) with methane as reagent gas. The sensitivity of the Exactive GC was tested by injecting low level solvent standards (in cyclohexane) prepared by a serial dilution of the two SCCP technical mixtures. SCCPs linearity and dynamic range was assessed for each SCCP technical mix (63% and 55.5%) using the following concentration range: 1 - 10000 pg/µL (in cyclohexane). Moreover, a SCCPs 55% chlorine standard was spiked with polychlorinated biphenyls to test the effect of various resolving powers on compound selectivity.

RESULTS: Disinfection by-products

Figure 1. Overlayed extracted ion chromatograms (m/z 126) of Milli-Q water spiked with natural organic matter (NL NOM) subjected to chlorination (red) and a control of untreated water (blue)
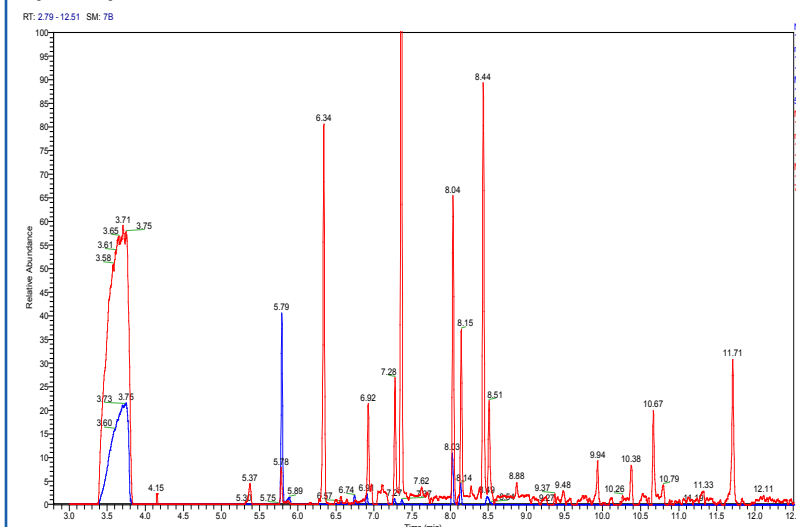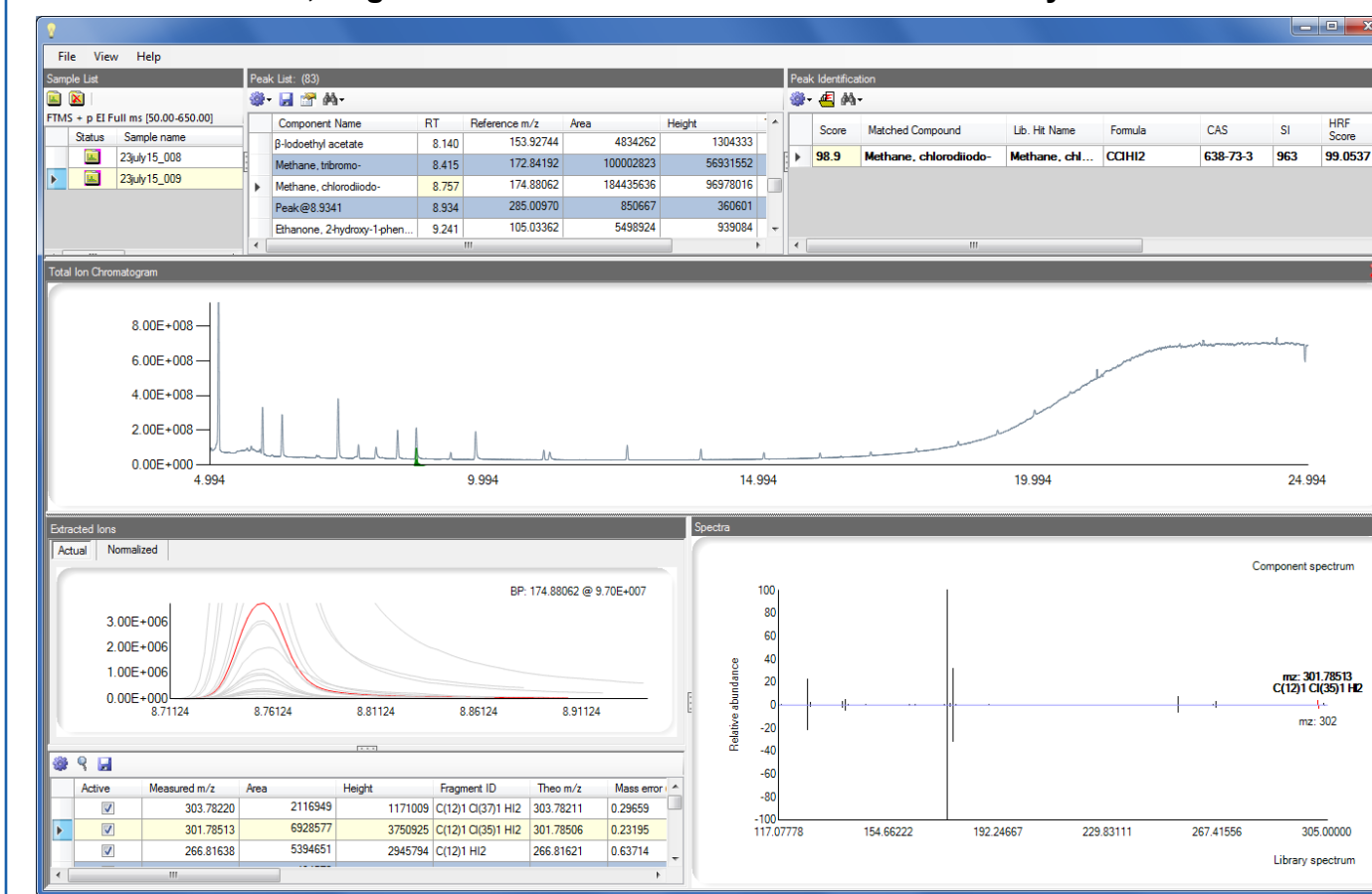

The DBP mixture concentrates obtained from the lab-scale chlorination and chloramination reactions were analysed in full-scan mode. An example of chromatographic separation is shown in Figure 1 below for untreated-control and chlorinated samples.

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 EI was processed in Thermo Scientific™ TraceFinder™ software for peak detection and spectral deconvolution using by compound identification using a 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 library match. Consequently, the confidence in compound identification is dramatically increased, as the analyst does not only rely in a library matching score, such as forward match.

An example of peak deconvolution in TraceFinder 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 NIST library, taking into account the forward search index (SI). In addition, a 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 $CHCl_2$ for chlorodiiodomethane. This resulted in a combined total score indicating the quality of match between this library hit and the deconvoluted measured spectrum.

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

Figure 3. TraceFinder browser showing chlorodiiodomethane identification based on NIST search index, fragment ions rationalization and mass accuracy information

Following the identification workflow described above, a total of eight different iodo-DBPs were confidently identified in the extracts analyzed (Table 1). Sample comparisons revealed that significantly higher levels of DBPs were observed in the chloraminated samples compared to the chlorinated extracts (Figure 4).

Table 1. Iodo-DBPs identified and confirmed in disinfected NL NOM waters.

Figure 4. Fold increase of iodo-DBPs detected and identified in chloraminated DBP mixture concentrates as compared to chlorinated ones
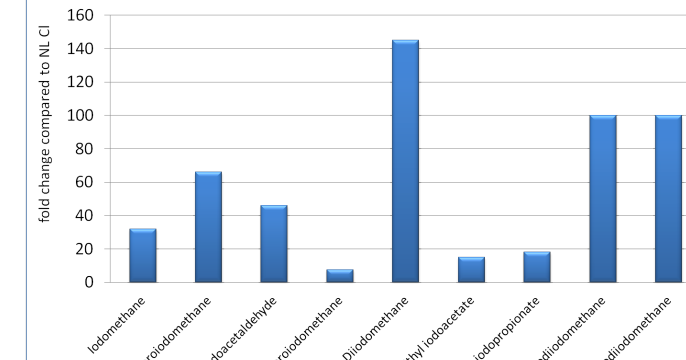
RESULTS: Short chained chlorinated paraffins

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 5.

Figure 5. High resolution accurate mass selectivity demonstrated for the C10-C13 63% technical mix acquired in NCI at 60k resolution. Examples of extracted ion chromatograms for individual homologues with various chlorination degrees are shown.

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 5.

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, $C_{11}H_{16}Cl_5$) 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 accurate 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). In order to achieve sub-ppm mass accuracy and the selectivity required for consistent separation and quantification of target compounds resolving powers of >30k are needed.

The sensitivity of the Exactive GC was tested by injecting low level solvent standards (in cyclohexane) prepared by a serial dilution of the two SCCP technical mixtures. 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 SCCP technical mixtures). In addition, the NCI data demonstrates that IDLs as low as 3 pg/µL can be obtained for individual homologue groups (Table 2).
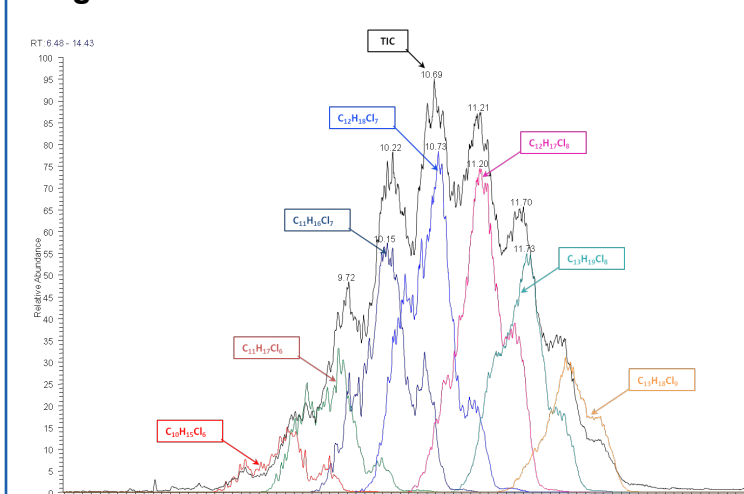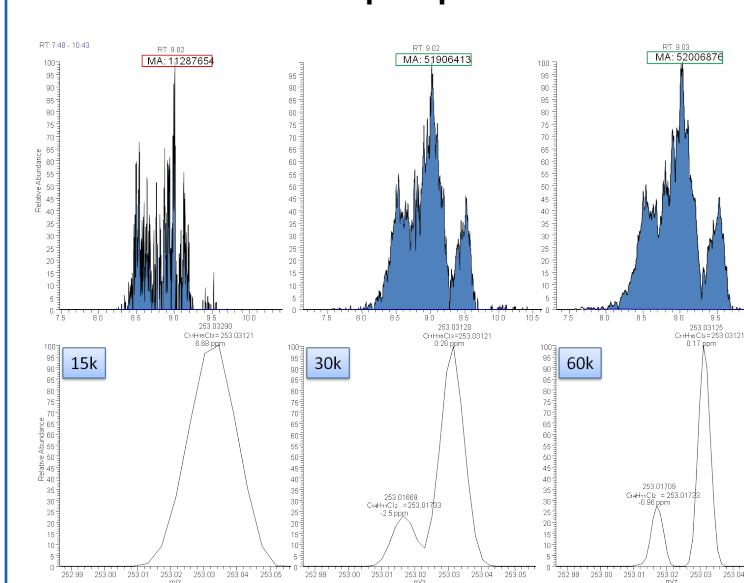
Figure 6. Selectivity enhancement by using narrow mass tolerance windows is possible at high resolving power. The effect of increasing resolving powers of the mass accuracy and peak area of a SCCP ion m/z 250.03121 is demonstrated for a 55% SCCPs sample spiked with PCBs.

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 example is given in Figure 7. The coefficient of determination was >0.99 indicating excellent linearity across this concentration range.

Table 2. Peal area repeatability of selected SCCP homologues. Data acquired in full-scan at 60k resolution using NCI using methane as reagent gas.

Inj. no	m/z 492.8546 ($C_{13}H_{16}Cl_9$)	m/z 458.8936 ($C_{13}H_{17}Cl_8$)
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 7. Example of SCCP linearity shown for m/z 346.92699 (±5ppm) $C_{10}H_{15}Cl_6$. Data acquired in NCI at 60k resolution

CONCLUSIONS

- A large number of peaks were detected in the water samples analyzed with higher concentrations of iodo-DBP in the chloraminated samples compared to chlorination treatments.
- The EI data was used for candidate compound identification using NIST. 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.
- Additionally, these preliminary results demonstrate that the GC-Orbitrap MS technology is a high potential solution for the detection and quantification of SCCPs with excellent sensitivity, linearity and selectivity and using an uncomplicated instrumental setup.
- Using NCI it is possible to selectively separate C10 alkanes chains with various chlorination degrees making quantification of homologues with similar CI 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.
- Taken together, 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.
- Moreover, 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

1. S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D. M. DeMarini, Mutat. Res. 636 (2007) 178-242.
2. S.D. Richardson, F. Fasano, J.J. Ellington, F.G. Crumley, K.M. Buettner, J.J. Evans, B.C.Blount, L.K. Silva, T.J. Waite, G.W. Luther, A.B. McKague, R.J. Miltner, E.D. Wagner, M.J. Plewa, Environ. Sci. Technol. 42 (2008) 8330-8338.
3. Postigo C., Cojocariu CI., Richardson SD., Silcock P., Barcelo D. 2015. Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters using Orbitrap-based gas chromatography-mass spectrometry, Anal. ChemDOI 10.1007/s00216-016-9794-3.

TRADEMARKS/LICENSING