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

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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.

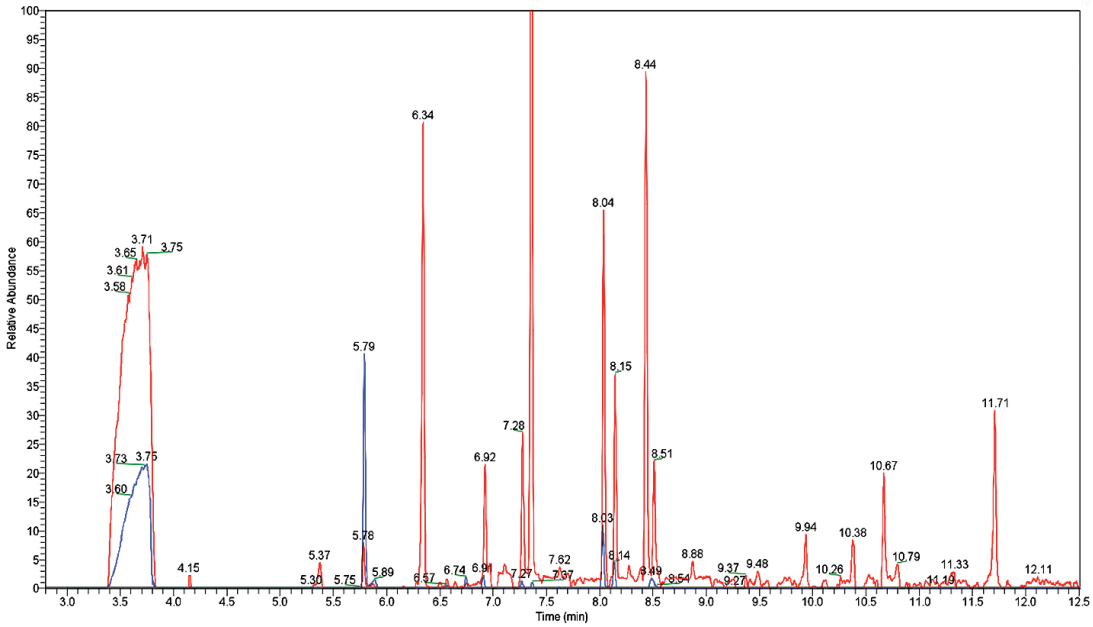


Figure 1. Overlayed extracted ion chromatograms (*m/z* 126.90392, iodine) of Milli-Q water spiked with natural organic matter (NL NOM) subjected to chlorination (red) and control of untreated water (blue) showing an increase in both the number and intensity of iodine-containing peaks in the chlorinated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

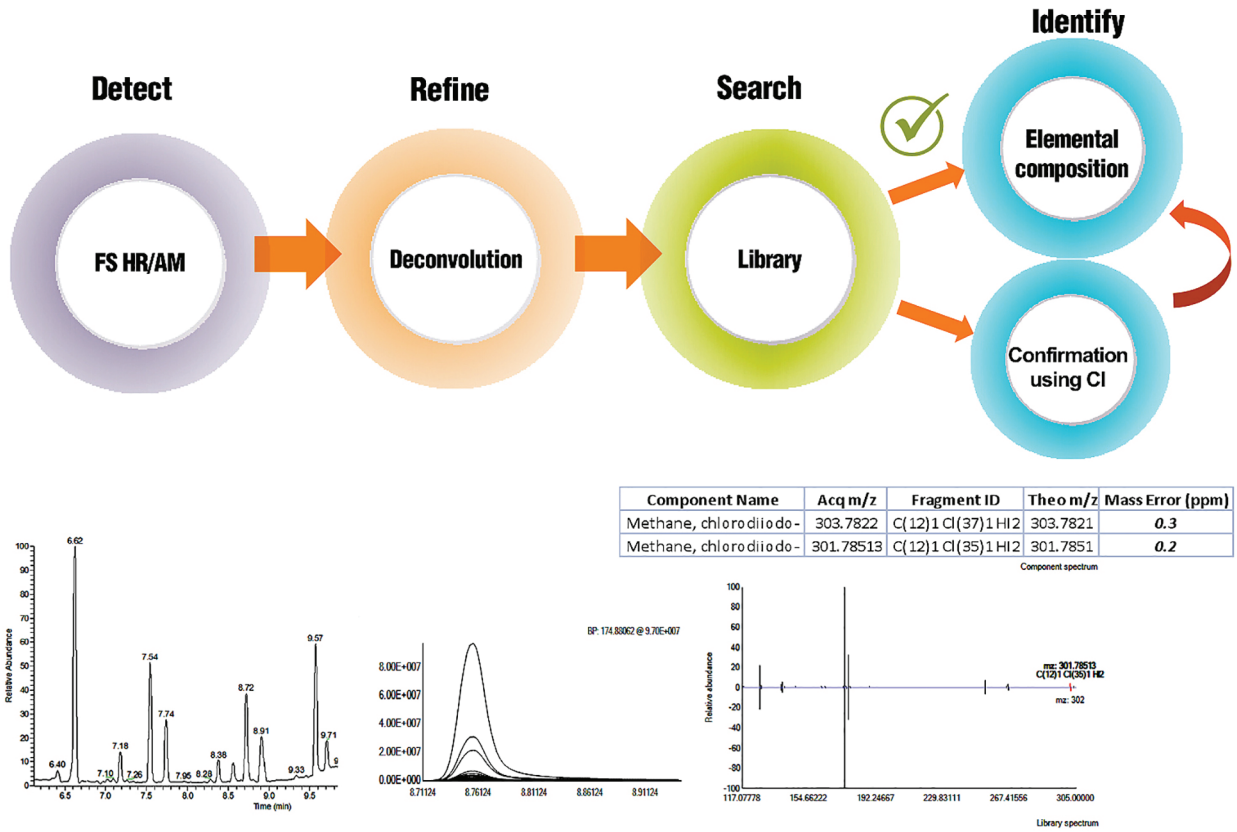


Figure 2. Compound discovery workflow used for iodo-DBPs peak detection with spectral deconvolution and tentative compound identification.

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.

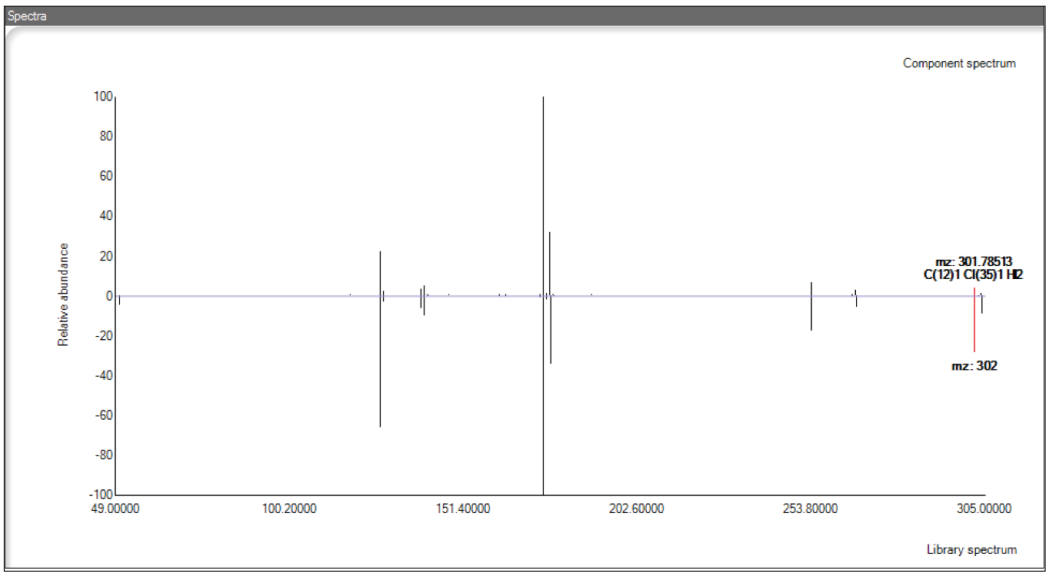


Figure 4. Ion mass spectrum, corresponding accurate masses (ppm) and elemental composition of chlorodiiodomethane (RT= 8.77 min) a) in the chloraminated NL NOM extract and b) MS library match. Data acquired in EI at 60,000 resolution (FWHM, at *m/z* 200). Annotated are the acquired fragment ions that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

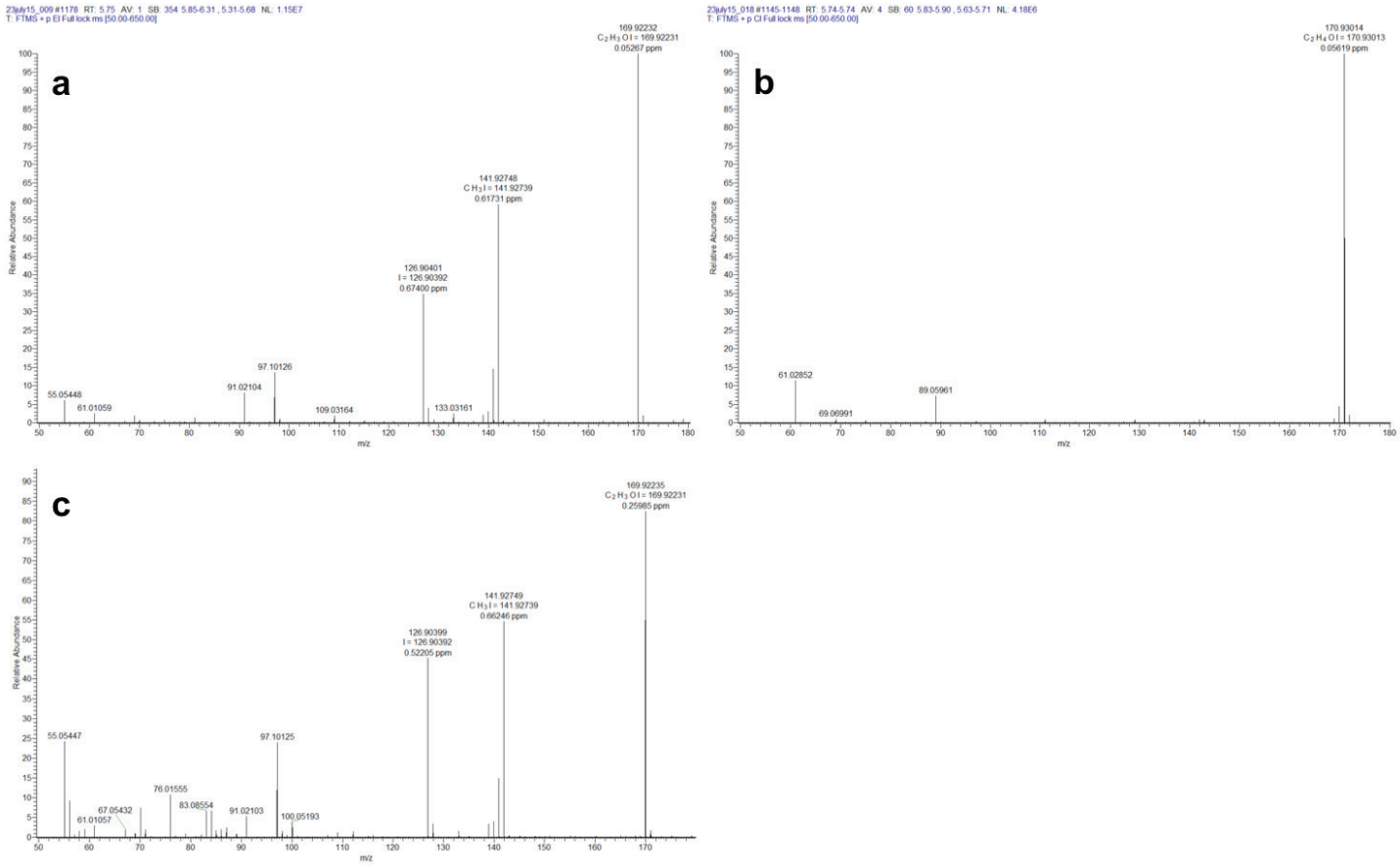


Figure 5. Confirmation of iodoacetaldehyde identification with authentic solvent standard (a) and NL treated samples (c) based on RT and mass accuracy measurements. Positive chemical ionization (PCI) mass spectrum (b) confirms mass of molecular ion $[M+H]^+$ with 0.06 ppm mass accuracy.

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.

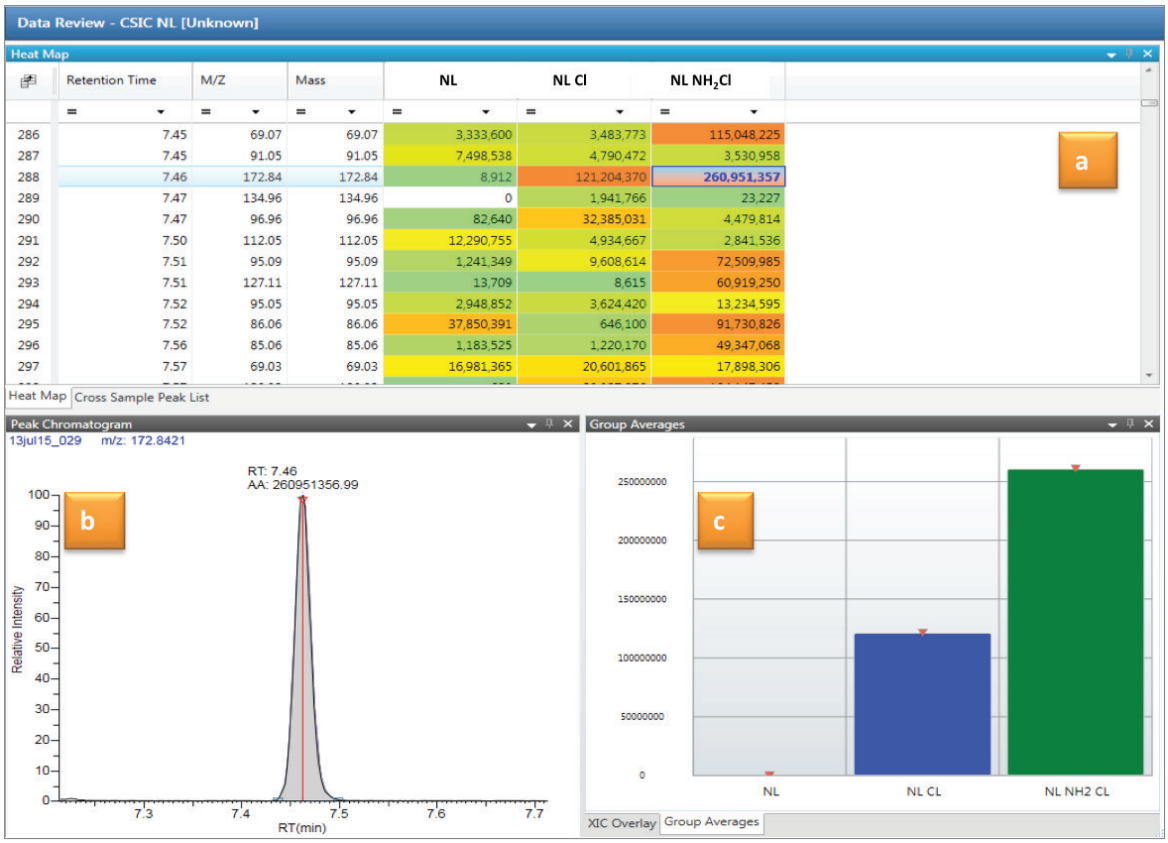
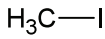
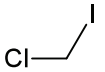
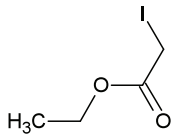
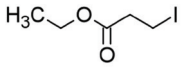


Figure 6. TraceFinder browser showing the heat map with the peak areas of detected peaks (a), and as an example, the increased concentration of a compound eluting at RT = 7.46 min, the corresponding extracted peak chromatogram (b), and the abundance of this chemical in the samples analyzed (c).

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).

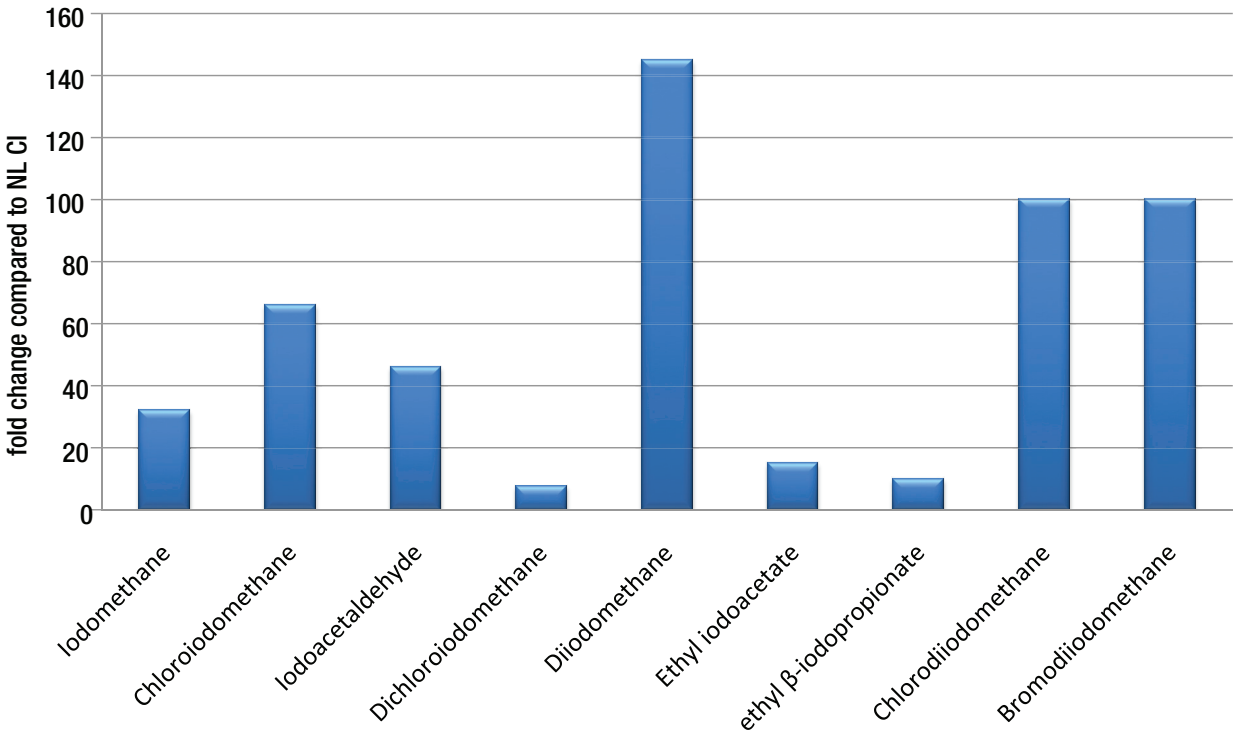


Figure 7. Fold increase of iodo-DBPs detected and identified in chloraminated DBP mixture concentrates as compared to chlorinated ones.

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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