WHITE PAPER

Simple determination of haloacetic acids (HAAs) in potable water with ion chromatography hyphenated to mass spectrometry

Haloacetic acids (HAAs) are commonly produced as disinfection byproducts (DBPs) from water treatment processes. Some HAAs are regulated by the authorities and have been classified as potentially carcinogenic. They have traditionally been analyzed by gas chromatography (GC), a technique that requires time-consuming sample extraction and derivatization, leading to higher costs per analysis.

Ion chromatography hyphenated to mass spectrometry (e.g., single or triple quadrupole MS systems) is a powerful tool that can handle many challenging analytical tasks such as measuring µg/L levels of HAAs in potable water samples. After separating the sample components via IC, mass selective detection guarantees peak identity with low detection limits. The inclusion of automated Metrohm Inline Sample Preparation (MISP) allows a variety of sample types to be readily analyzed without need for extensive manual laboratory work. Automation of sample preparation steps besides the analysis results in more reliable and reproducible data.

This White Paper explains the benefits of using this hyphenated technique for the accurate measurement of HAAs in potable water.

Metrohm

INTRODUCTION TO ION CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

Ion chromatography (IC) tackles difficult separation problems using the interactions between ionic species in liquid samples and a stationary separation column, typically utilizing conductivity detection. Mass detection accomplished with a mass spectrometer as a secondary independent detector (e.g., MS, MS/MS) for IC records the mass-to-charge ratios (*m/z*) of selected analytes of interest. As such, mass detection confirms the identity of compounds present in challenging matrices to ensure that the correct results are obtained without question. Co-eluting components can be quantified and detection limits are considerably improved.

High-performance liquid chromatography mass spectrometry (HPLC-MS) is a well-established analysis technique. However, ionic compounds are difficult to separate with conventional HPLC-MS, but this can be achieved easily by using IC-MS [**1**]. For these analytes, IC-MS is able to succeed with specific columns tailored for ion separation. An inline suppressor reduces the ion load from the mobile phase to a minimum in order to optimize signal-to-noise ratios for both the conductivity and the MS detectors.

IC-MS is a robust and easy-to-use technique for the determination of analytes such as inorganic anions, organic acids, haloacetic acids, oxyhalides, or alkali and alkaline earth metals [**2**]. Adding **Metrohm Inline Sample Preparation (MISP)** to the analysis allows not only water samples, but also chemicals, organic solvents, or post-explosion residues to be readily analyzed [**3**].

CHARACTERISTICS OF MASS DETECTION FOR SMALL IONIC COMPOUNDS

With IC, many analytes can be detected via conductivity, although other common detectors can be used (e.g., UV/VIS, amperometric detection). Identification is done by comparing the retention time of defined peaks in a chromatogram with those of prepared standards. This can be challenging for samples with multiple components or with extreme concentration differences between matrix and analyte. With a mass detector used in series, low detection limits and correct peak identification are assured by correlating the conductivity signal with the respective mass-to-charge signal. Hyphenation of IC with a mass detector results in substantial broadening of the application field [**4**].

Molecular ions are typically analyzed using single quadrupole mass spectrometers (**Figure 1**). High-end mass spectrometers such as triple quadrupoles have even greater sensitivity and can elucidate structural details with fragmentation studies.

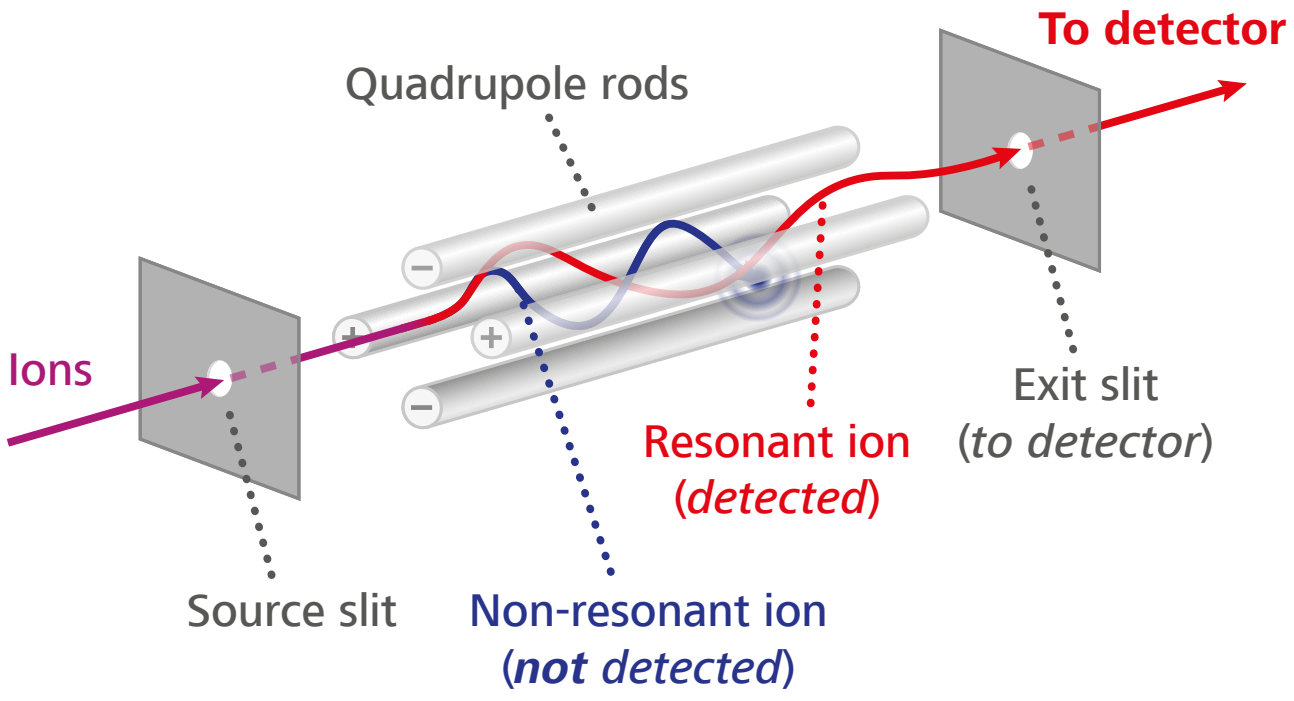

Figure 1. Illustrated schematic of the working principle of a single quadrupole mass spectrometer.

HYPHENATION OF IC AND MS DEVICES

An IC can be coupled to any MS type from any instrument supplier (**Figure 2**). Synchronization of both instruments typically utilizes a remote box and a suitable cable connection to notify the MS detector to start recording once the sample is injected.

Regarding the hardware connection, a simple capillary connection between the conductivity detector and the MS detector is sufficient. The conductivity detector is a non-destructive detector with little dead volume (>1 µL) and its outlet capillary (a PEEK capillary with 0.25 mm inner diameter) is typically connected to either the injection valve of the MS, or directly to its source inlet.

Further additions may improve the application. A splitter can optimize the sample flow rate that reaches the MS for optimal signal-to-noise ratios. A switch valve can be used to channel the flow into the MS only when elution of analytes of interest is expected. The flow is diverted to the waste when matrix peaks elute, during instrument equilibration, and automatic sample preparation, in order to keep contamination of the MS to a minimum. Some applications require reagent addition (e.g. organic solvent) by post-column reaction (PCR) prior to the MS analysis, or even need an additional suppressor module to adjust the pH value or remove matrix ions to avoid signal suppression.

– GENERAL APPROACH FOR ALL MS TYPES

If there is no common software, both instruments are operated via their own software. The sample tables therefore must be copied to both softwares, and data evaluation is typically done in the software of the respective instrument (e.g., processing of the conductivity signal in the IC software and of the MS signals in the MS software). This approach is often used for high-end MS analysis with dedicated MS software.

For example, the IC is operated with MagIC Net from Metrohm, and the MS is controlled by software from Agilent (MassHunter™), Waters (MassLynx™) , or AB Sciex (Analyst®). These flexible and high performance setups unleash their full potential in development laboratories and research institutes.

The operation of such instrumentation requires trained personnel. Otherwise it can be challenging to work under full compliance since the complete combination is controlled by two independent softwares.

– IC DRIVERS FOR ONE COMMON SOFTWARE

Native IC drivers allow the control and operation of the IC equipment in the same software as the MS

Figure 2. Synchronization of IC is possible with MS instruments from any supplier.

(including autosamplers and high precision burettes like Metrohm Dosinos). One software for both IC and MS enables user-friendly handling and ensures robust operation. Software trainings take less time since only one software is involved. **Data integrity and full compliance are guaranteed.**

Metrohm offers solutions for **Waters Empower™ 3** and **Agilent OpenLab CDS** (Metrohm IC Driver for Empower™ 3, Metrohm IC Driver for OpenLab CDS).

DETERMINATION OF HALOACETIC ACIDS IN POTABLE WATER WITH IC COUPLED TO MS

Chlorine (in various forms) is widely used as a disinfectant for municipal water systems. Such water treatment processes can form chlorine disinfection byproducts (DBPs) in trace (µg/L) amounts, including haloacetic acids (HAAs) [**5,6**]. These HAAs are thought to pose potential cancer risks after continued exposure to high concentrations, although no definitive evidence of a link has been identified as of yet [**7**]. As a health precaution, various global agencies including the US Environmental Protection Agency (EPA) have defined a maximum threshold of HAA content in municipal waters (60 µg/L) which requires extremely low detection limits for the individual haloacetic acids [***8***]. More recently, the European Parliament revised the Drinking Water Directive by defining the limit of the sum of nine HAAs as 60 µg/L [**9**]. Thus, interest in measuring trace concentrations of HAAs in municipal water supplies has grown significantly [**10**].

– ACHIEVE THE LOWEST DETECTION LIMITS WHEN COUPLING IC TO HIGH-END MS

To address the need of measuring various haloacetic acids at µg/L levels in municipal water samples, a robust method was developed using IC-MS/MS which conforms to US EPA Method 557 [**11**]. The benefit of using MS/MS with a triple-quadrupole mass spectrometer over using MS with a single quadrupole mass spectrometer is the ability to avoid potential interferences while achieving lower detection limits and enhanced selectivity. Other analytical methods require complex and labor-intensive derivatization of the samples to achieve detection. In the developed IC-MS/MS method, water samples are spiked with known amounts of internal standards and analyzed directly without the need for derivatization, cleanup, or preconcentration steps. This saves a considerable amount of time per sample, increasing throughput and lowering operating costs. An advantage of using a Metrohm ion chromatograph for this task is that it allows the addition of organic solvent directly into the mobile phase (eluent) to not only enhance desolvation of the analytes in the electrospray ionization (ESI) interface, but also to improve chromatographic separation.

An application study of nine HAAs plus dalapon and bromate was performed with IC-MS/MS using a Metrohm ion chromatograph connected to an Agilent 6470 Triple Quadrupole LC/MS Mass Spectrometer (**Table 1**). These compounds were separated using a binary gradient on a Metrosep A Supp 7 - 250/4.0 separation column.

After suppression, the analytes were transferred to the mass spectrometer and selected MRM (multiple reaction monitoring) transitions were used for quantification (**Figure 3**). The calibration for this study covered a concentration range of 1–500 µg/L with linear regression. The chromatographic software used was MagIC Net, and the Agilent Mass Hunter™ software was used to operate the MS in this situation.

The performance of the method was demonstrated by measuring recoveries (87–127%) of a spiked water sample (**Table 2**). In addition, municipal water samples of the Tampa Bay area (Florida, USA) were also analyzed for their HAA content. These samples contained less than 60 µg/L total HAAs, which is the critical limit according to the US EPA guidelines.

Table 1. Retention times and MS/MS acquisition parameters for nine haloacetic acids, dalapon, and bromate.

Analyte	Retention time [min]	Multiple reaction monitoring transition [amu]
Bromate	9.14	126.9 → 110.8
Monochloroacetic acid (MCA)	9.38	93.0 → 35.0
Monobromoacetic acid (MBA)	9.69	137.0 → 79.0
Monoiodoacetic acid (MIA)	10.05	185.0 → 127.0
Dichloroacetic acid (DCA)	11.24	127.0 → 83.0
Bromochloroacetic acid (BCA)	11.77	173.0 → 80.9
Dichloropropionic acid (Dalapon)	11.80	141.0 → 97.0
Dibromoacetic acid (DBA)	12.46	216.8 → 78.9
Trichloroacetic acid (TCA)	16.74	117.0 → 34.9
Bromodichloroacetic acid (BDCA)	18.17	163.0 → 81.0
Dibromochloroacetic acid (CDBA)	20.03	206.7 → 79.1

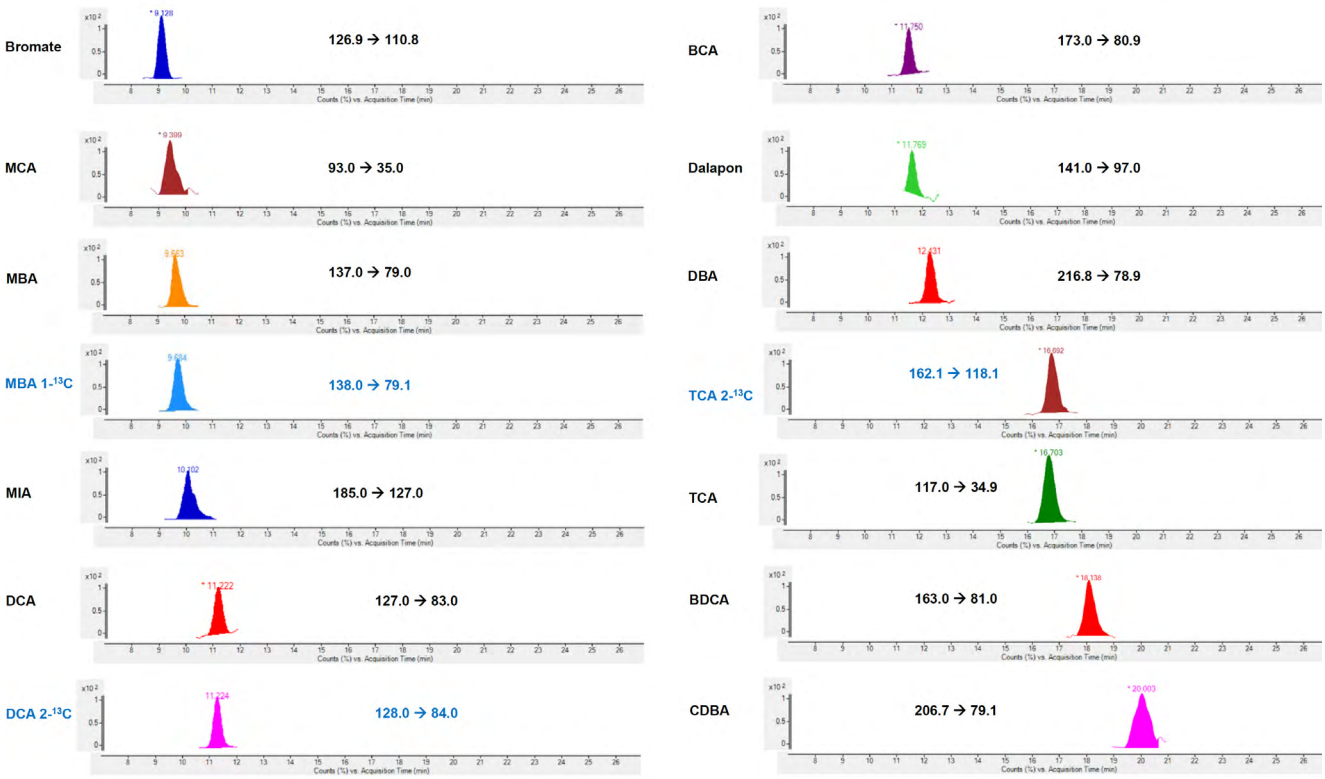

Figure 3. Mixed standard containing 50 µg/L of each analyte of interest and 100 µg/L of each internal standard, separated on a Metrosep A Supp 7 - 250/4.0 (eluent: potassium hydroxide/sodium carbonate/acetonitrile gradient, flow rate 0.7 mL/min, column temperature 45 °C, injection volume 100 µL). Analytes are labeled in black and internal standards are labeled in blue.

To tune and optimize the MS, an additional Dosino was used to supply a steady flow of analyte solution (0.2 mg/L) directly into the ESI source at a rate of 0.5 mL/min. In this way, the MS source settings could be optimized for each analyte in an automated way without chromatographic separation.

Diversion of the eluent flow to waste was performed for the inital seven minutes and from 27 to 28.5 minutes following injection. This diversion prevents detection of unretained compounds or non-targeted compounds, therefore minimizing contamination to the MS source and reducing the frequency of MS source cleaning.

Table 2. Municipal water sample spiked with 25 µg/L of each listed analyte and analyzed five times.

Analyte	Avg. Conc. [µg/L]	RSD [%]	Expected Conc. [µg/L]	Recovery [%]
Bromate	39.4	4.5	35.0	112
MCA	29.5	9.6	24.8	119
MBA	25.6	13.3	23.9	107
MIA	46.2	6.7	36.5	127
DCA	24.8	2.2	24.0	103
BCA	17.6	2.8	18.1	97
Dalapon	25.8	4.5	24.7	104
DBA	24.7	5.7	25.1	99
TCA	22.9	6.0	24.8	92
BDCA	25.8	4.9	24.1	107
CDBA	21.6	8.7	24.8	87

– USING INSTRUMENTS FROM OTHER MANUFACTURERS

The same application was performed with the instrument combination of Metrohm IC and the AB Sciex QTRAP 6500+. Chromatographic separation was achieved with the same conditions as before (**Figure 3**) and parameters for MS detection were adapted to the instrument. Bromate, dalapon, and ten HAA compounds were quantified in a range of 0.2 µg/L, or even 0.02 µg/L to 200 µg/L, depending on the analyte. The chromatographic separation with this setup is shown in **Figure 4**, using Analyst® software from AB Sciex.

Monofluoroacetic acid (MFA), difluoroacetic acid (DFA), monoiodoacetic acid (MIA), and trifluoroacetic acid (TFA) were additionally investigated but not quantified. Spiking tests with tap water (**Figure 5**) and bottled water (**Figure 6**) showed the accuracy and the robustness against matrix effects of this method.

In general, Metrohm ion chromatographs can be coupled to any MS detector. In a study about suspect screening of halogenated carboxylic acids in drinking water, a Metrohm IC was coupled to an Exactive Orbitrap from Thermo Fisher Scientific [**12**]. There, a flexible IC-HRMS method was developed for the purpose of detecting a wide *m/z* range of organic anions with detection limits in the µg/L range, with a particular focus on measuring halogenated carboxylic acids in drinking water.

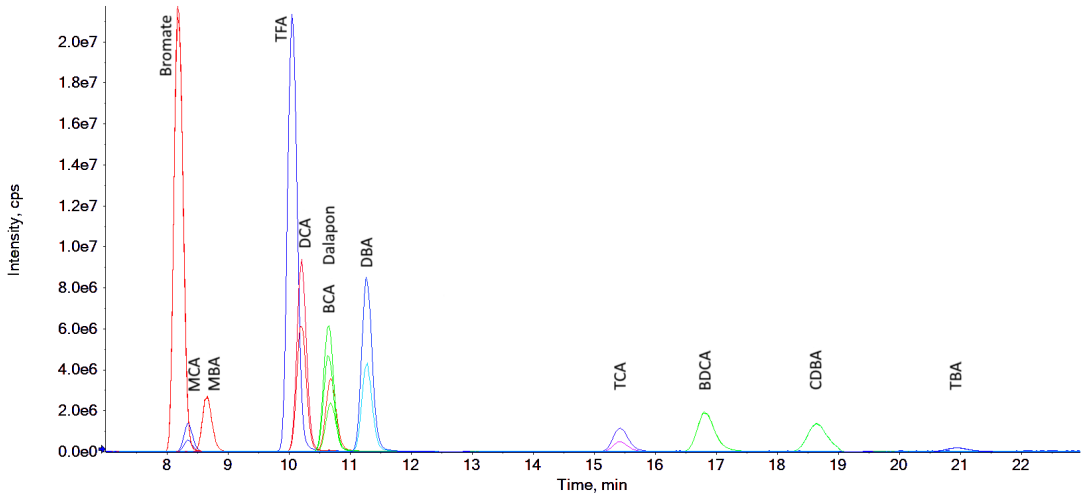

Figure 4. Chromatographic separation of ten HAA compounds (including TFA and TBA), bromate, and dalapon (200 µg/L of each analyte, 100 µL injection volume) on a Metrosep A Supp 7 - 250/4.0 column (eluent: sodium hydroxide/sodium carbonate/acetonitrile gradient, flow rate 0.8 mL/min, column temperature 45 °C).

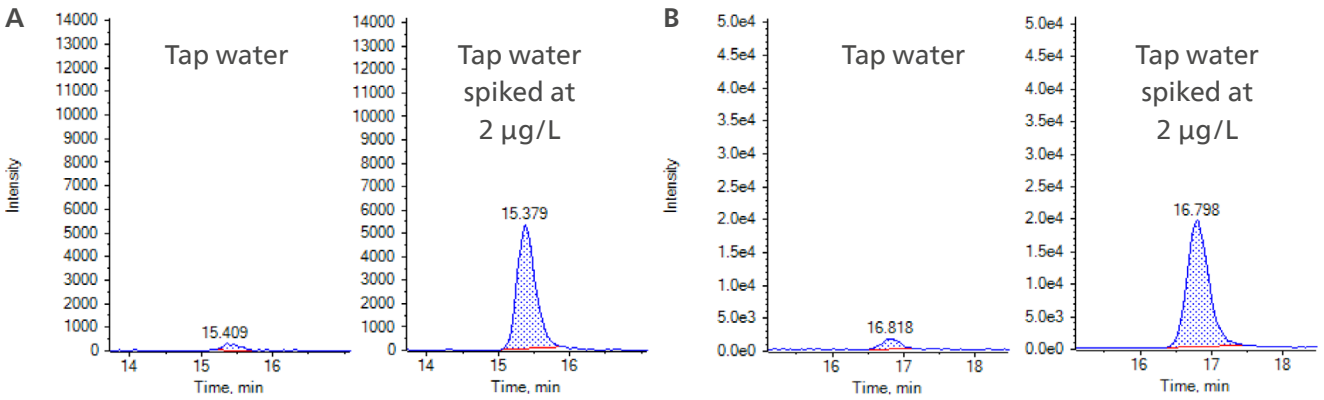

Figure 5. Example chromatograms for TCA (A) and BDCA (B) spiked in tap water (2 µg/L of each compound, 100 µL injection volume). The software used was Analyst® from AB Sciex.

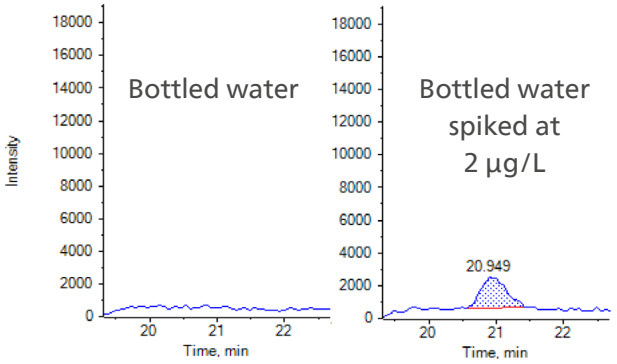

Figure 6. Example chromatograms for 2 µg/L tribromoacetic acid (TBA) spiked in bottled water (100 µL injection volume). The software used was Analyst® from AB Sciex.

– COUPLING IC TO SINGLE QUADRUPOLE MS: ROBUST AND EASY TO USE

The current ISO norm (ISO 23631:2006) recommends using GC-MS to measure six haloacetic acids in water [**13**]. An upcoming drinking water directive draft may indicate the use of other analytical techniques (e.g., IC-MS or IC-MS/MS) for the purpose of measuring nine haloacetic acids with concentrations that must not exceed 5 µg/L individually, and their combined concentration must not exceed 60 µg/L [**9**].

As part of an interlaboratory study, it was proven that hyphenation of a Metrohm IC with a single quadrupole mass spectrometer (Waters SQ Detector 2) was suitable for this application (**Table 3**) [**14**]. Empower™ 3 software was used to control the entire setup, which makes the application compliant and easy to perform for any operator.

Separation of nine HAAs (MCA, MBA, DCA, BCA, DBA, TCA, BDCA, CDBA, and TBA) in different water matrices was accomplished within 35 minutes by using a Metrosep A Supp 5 - 250/2.0 column in combination with a Metrosep Supp 10/4.0 guard column. The 2 mm microbore column is well-suited for MS detection with respect to the lower flow rates it requires. For this separation column, the optimal eluent flow rate is 0.2 mL/min which is also perfect for MS detection purposes. The additional guard column improved the separation between MCA, MBA, and chloride. Lowering the column temperature to 10 °C further improved the separation. After suppression, the conductivity signal and selected ion recordings were collected. Corresponding ^{13}C standards were used to correct quantification to avoid any matrix effects. It was possible to detect 5 µg/L or less of the HAAs in all tested sample matrices with this setup (**Figures 7** and **8**).

Table 3. Estimated limit of quantification (LOQ) of several HAAs in ultrapure water (from S/N = 10 extrapolation).

Analyte	Estimated LOQ [µg/L]
MCA	0.15
MBA	0.15
DCA	1.5
BCA	2
DBA	0.5
TCA	5
BDCA	5
CDBA	5
TBA	5

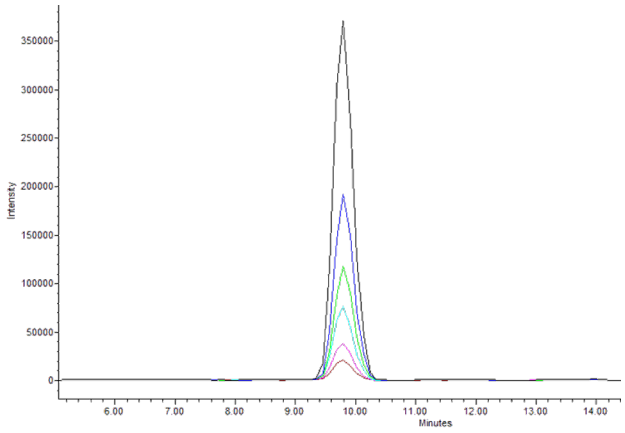

Figure 7. Sample of Evian water spiked with MCA (*m/z* 93; spiking levels: 5, 10, 20, 30, 50, and 100 µg/L), separated on a Metrosep A Supp 5 - 250/2.0 column (injection volume 100 µL, eluent: potassium hydroxide/sodium carbonate/acetonitrile gradient, flow rate 0.2 mL/min, column temperature 10 °C). The software used was Empower™ 3.

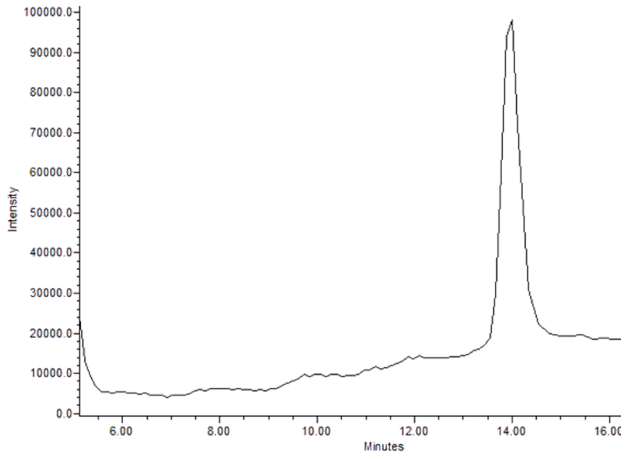

Figure 8. Example chromatogram of DBA (*m/z* 217; 25 µg/L), separated on a Metrosep A Supp 5 - 250/2.0 (injection volume 100 µL, eluent: potassium hydroxide/sodium carbonate/acetonitrile gradient, flow rate 0.2 mL/min, column temperature 10 °C). The software used was Empower™ 3.

A similar approach was accomplished when using a single quadrupole MS from Agilent. LCMRL (lowest concentration minimum reporting level) values below 0.4 µg/L were reached for six HAAs in this case [**15**]. At the time of the study, two software packages were used (one for chromatography and one for mass spectrometry). Today, a **single software solution** with Agilent OpenLab CDS for both IC and MS guarantees convenient handling and full traceability.

– MEASUREMENT OF TRIFLUOROACETIC ACID (TFA) IN TAP WATER WITH IC-MS

Trifluoroacetic acid (TFA) levels in the environment have increased over time due to atmospheric photo-oxidation of fluorinated refrigerants and propellants (e.g., polyfluorinated chemicals, also known as PFAS) or from industrial discharge [**16**]. Furthermore, TFA forms a metabolite of fluorine-containing herbicides. Trifluoroacetic acid is omnipresent—found even in the remote arctic [**17**]. Therefore, TFA is considered as an environmental contaminant and is associated with adverse risks for human health, however, the exact reaction mechanisms are not yet completely understood. Limits for TFA are not regulated in many countries, despite ongoing discussions to do so. Some countries do give recommendations, for instance the German Federal Environmental Agency allows a daily intake of 60 µg/L TFA in drinking water (assuming an average consumption of 2 L per day) [**18**].

This study demonstrates the determination of TFA by using IC-MS. Ions were separated isocratically on a Metrosep A Supp 17 - 100/4.0 separation column equipped with a Metrosep RP 2 Guard/3.5. Chemical suppression ensured a low background conductivity for enhanced detector sensitivity. The TFA anion (*m/z* 113) and its fragment $F_3C\cdot$ (*m/z* 69) were monitored with a single quadrupole MS (Waters SQ Detector 2).

Different water matrices were simulated by mixing up to 200 mg/L each of chloride, nitrate, and sulfate. Even in samples with the highest matrix load, a concentration of 0.5 µg/L TFA was determined well above the quantification limit with an injection volume of 20 µL (**Figure 9**). The water matrices did not interfere with the signal to a great extent, because chromatographic resolution of TFA and the matrix peaks was high enough. Even nitrate, eluting before TFA, did not significantly quench the signal.

Thus, the presented application for TFA determination in tap water works well for the sub-µg/L concentration range with the coupling of single quadrupole MS. In the case that even lower detection limits must be reached, then high-end MS/MS detectors would be required. TFA analysis with a similar concentration range is also possible with re-injection analysis on a simple IC system (without requiring MS) [**19**].

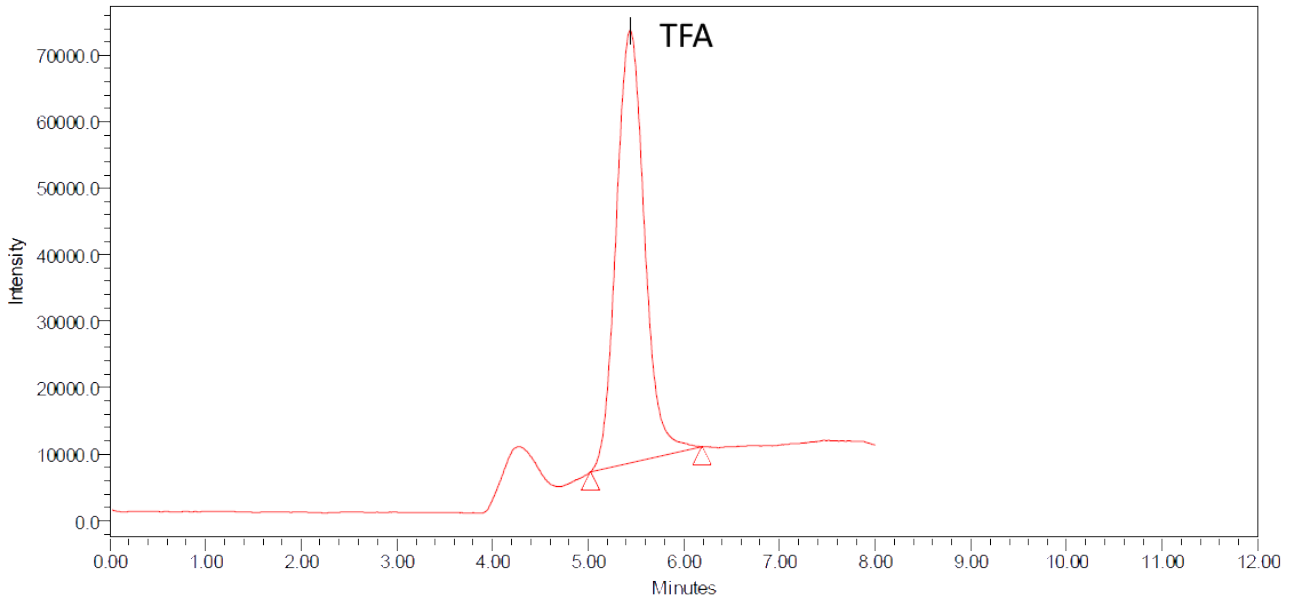

Figure 9. Chromatogram showing the selected ion recording of TFA (*m/z* 113; 0.5 µg/L) in a matrix containing 200 mg/L each of chloride, nitrate, and sulfate, separated on a Metrosep A Supp 17 - 100/4.0 column (injection volume 20 µL, eluent: 8.5 mmol/L sodium carbonate, 10% (v/v) acetonitrile, flow rate 1.0 mL/min, column temperature 30 °C). The software used was Empower™ 3.

SUMMARY

Liquid chromatography is a powerful analytical tool in the laboratory, especially for challenging samples which contain multiple analytes in highly varied concentrations. For this reason, the unique combination of IC with MS is becoming more and more popular for applications involving ionic and polar molecules.

Determining haloacetic acids in potable water is important to ensure the safety of the water supply and therefore the health of the population. When it comes to measuring HAAs at low concentrations (µg/L), Metrohm IC is the ideal analytical solution for their separation. With the ability to couple to any MS detector, a large selection of separation columns, and the many automated Metrohm Inline Sample Preparation (MISP) options, robust ion chromatography for trace analysis has never been so easy.

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Further related Metrohm literature

A strong combination – Coupling of Metrohm ion chromatography and mass spectrometry **8.000.5250**

Metrohm meets Empower 3 – Ion chromatography with the Chromatography Data Software (CDS) from Waters **8.102.5004**

IC Driver for Agilent OpenLab CDS – A perfect combination: Metrohm Ion Chromatography and OpenLab CDS Software **8.102.5007**

IC-ICP/MS with Metrohm – Coupling of Metrohm ion chromatography and mass spectrometry with inductively coupled plasma **8.000.5241**

Metrohm Application Finder: Selection of IC-MS Applications

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