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Suspect screening of halogenated carboxylic acids in drinking water using ion exchange chromatography – high resolution (Orbitrap) mass spectrometry (IC-HRMS)

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Abstract

Retrospective in silico screening of analytical data for the identification of new or emerging disinfection by-products in drinking waters could be useful to assess quality and potential hazards, as well as help implement mitigation procedures more rapidly. Herein, the first study coupling ion exchange chromatography (IC) with high resolution mass spectrometry (HRMS) for the determination of halogenated carboxylic acid disinfectant by-products is reported. Separation was achieved using a Metrohm A Supp 5 column and a Na2CO3/NaHCO3 gradient eluent from 1/0.31 to 10/3.1 mM. A variety of solid phase extraction (SPE) sorbents were tested for added selectivity to organic ions and Isolute ENV+ cartridges were selected because of their best overall extraction performance. Method LODs were in the μg L-1 concentration range, with $R2 \geq 0.99$ for all the analytes, and isobaric ions could be easily discriminated using HRMS. The method was applied to municipal drinking water. Targeted quantitative analysis revealed the presence of 10 haloacetic acids at levels not exceeding the limits set by WHO and USEPA. Furthermore, suspect screening for additional halogenated carboxylic acids via retrospective HRMS data analysis also indicated the presence of other iodinated HAAs and chlorinated propionic acids, of which one (i.e. monochloropropionic acid) is discussed here for the first time. Most importantly, several potential suspects could be eliminated from further consideration through HRMS data analysis alone. To our knowledge, this represents the first time that a retrospective IC-HRMS screen of halogenated carboxylic acids in drinking water has been reported.

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1. Introduction

Disinfection is a crucial step in the treatment of drinking water, where methods such as chlorination and ozonation are currently employed in several countries worldwide [1, 2]. Such methods have nonetheless been linked to the formation of disinfection by-products (DBPs) which have been reported to pose long-term health risks for humans [3, 4]. Examples are oxyhalides (such as chlorate and bromate), trihalomethanes (THMs) and haloacetic acids (HAAs). Monitoring of DBPs has thus become very important to ensure drinking water quality and safety, with several regulatory bodies setting or suggesting acceptable maximum concentration levels (MCLs). For dissolved organic DBPs such as HAAs, for example, the World Health Organization (WHO) have set guidelines for MCLs of 3 specific HAAs, i.e. chloro-, dicholoro- and trichloroacetic acid, at 20, 50 and 200 µg L-1, respectively [5]. In the American context, the United States Environmental Protection Agency (USEPA) set a MCL of 60 µg L-1 for the combined concentration of 5 HAAs (namely, chloro-, dichloro-, trichloro-, bromo- and dibromoacetic acid), with the goal that dichloroacetic acid should never to be present and that trichloroacetic acid should not amount to more than 20 µg L-1 [6].

Ion exchange chromatography (IC) is nowadays recognised as a valuable method for determination of trace HAA residues and it holds several benefits over other techniques such as gas chromatography (GC) and capillary electrophoresis (CE). These mainly lie in its compatibility with aqueous samples/extracts and its ability to separate small charged non-volatile inorganic and organic compounds; features which often negate several preparation steps (e.g. derivatisation for GC-MS) and overall make the entire procedure more rapid and straightforward. Suppressed conductivity detection (SCD) has been the detection mode traditionally used with IC. Nonetheless, IC coupled to mass spectrometry (MS) has been used in recent works as a confirmatory technique based on its better selectivity and sensitivity. Several types of mass analysers have been applied to the determination of HAAs in drinking water, usually after electrospray ionisation (ESI) or heated electrospray ionisation (HESI). These have included single MS in full scan and selected-ion monitoring (SIM) modes [7, 8], as well as tandem MS with selected-reaction monitoring (SRM) [9, 10].

Despite its excellent performance, the main limitation of published IC-MS methods to date is their requirement for pre-selection of ions meaning that all other ionic components of the matrix are not measured. In many cases, this prohibits the possibility of retrospectively analysing data after acquisition for compounds not included in method optimisation; a procedure which can be useful, for example, to identify other compounds of potential interest in the specific case and/or characterise new or emerging species (i.e. suspect screening). Suspect screening is of high interest for the development of modern monitoring methods, as it is supposed that only a fraction of the contaminants that are

present in drinking water have currently been identified [11]. Previous work used IC coupled to an ion trap mass analyser which allowed some degree of retrospective analysis of data, but high assurance identification of new compounds was not possible due to lower resolution and mass accuracy [7]. In order to enable higher quality suspect screening for new/additional compounds, high resolution mass spectrometry (HRMS) is an obvious potential solution. Indeed, HRMS detectors (and especially Orbitrap mass analysers) can perform fast measurements of relatively large m/z ranges with very high resolving powers (up to 140,000 FWHM) and mass accuracy (< 5 ppm). Benefits include the possibility of acquiring full-scan data with minimised interference from major non-isomeric compounds (including isobaric compounds), as well as the possibility to perform tandem MS as needed and in several cases infer elemental composition directly. Hyphenation of HRMS with different liquid-based chromatographic systems have been reported in many successful applications across analytical chemistry for suspect screening, often in highly complex matrices [12, 13]. However, only a few IC-HRMS works have been described [14-16] and, to our knowledge, no work dealing with the quality assessment of drinking water is currently available.

The aim of this study was to develop a new, flexible IC-HRMS method for detection of a wide m/z range of organic anions and assess its potential for retrospective suspect screening of trace emerging contaminants in drinking water. Given that new contaminants are being identified on an on-going basis [17], special focus has been placed here on suspect screening for longer chain halogenated organic acids, whilst maintaining the capability for quantitative targeted analysis of regulated or known HAA-type DBPs. The implemented method exploited IC coupled to Orbitrap HRMS technology and was optimised on 10 selected HAAs, which possess different degrees and type of halogenation and interaction strengths with anion-exchange resins. Solid phase extraction (SPE) sorbents and conditions were selected to maximise sensitivity for organic species in particular to provide a broadly applicable suspect screening method. The analytical performance was assessed before application to real samples. To our knowledge, this represents the first time that a full-scan IC-HRMS method has been developed for the analysis of trace organic contaminants in drinking water, and applied in a retrospective suspect screen for preliminary identification of new/additional halogenated carboxylic acids.

2. Experimental

2.1. Materials

Water used throughout this work was of Milli-Q grade with a specific resistance of 18.2 MΩ cm-1 and obtained from a Synergy UV ultra-purification system (Millipore Corp., Bedford, USA).

Methanol was of HPLC grade and was obtained from Honeywell (Bracknell, UK). The 10 reference HAAs were monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), trifluoroacetic acid (TFAA), bromochloroacetic acid (BCAA), chlorodibromoacetic acid (CDBAA), bromodichloroacetuc acid (BDCAA) and chlorodifluoroacetic acid (CDFAA). Standards were obtained from Sigma-Aldrich (Gillingham, UK) and were of analytical grade (≥ 98 %). Stock solutions were prepared for each HAA at a concentration of 1000 mg L-1 in Milli-Q water and stored in the dark at 4 °C in a refrigerator. Working solutions were prepared fresh daily by further dilution of stock solutions and stocks were re-prepared on a monthly basis. Sulfuric acid served an IC suppressor regenerant and was obtained at analytical grade (98 %) from VWR Chemicals (Lutterworth, UK). An aqueous buffer of sodium carbonate and sodium bicarbonate (Na2CO3/NaHCO3) was used as eluent. Sodium carbonate was obtained from Sigma-Aldrich, and sodium bicarbonate from BDH Chemicals Ltd. (Poole, UK). Both were of analytical grade (≥ 99.5 %).

2.2. IC conditions

Experiments were performed on a 850 Professional IC equipped with a 858 Professional Sample Processor (both from Metrohm, Herisau, Switzerland). This instrument was fitted with two high pressure pumps allowing gradient applications, a conductivity detector Metrohm IC Professional Detector, an ion trap Metrohm Metrosep A Trap 1 (100 x 4 mm, 5.0 µm particle size) providing eluent purification before the injection valve, and a 3-step column suppressor (Metrohm MSM Rotor A). The latter was chemically regenerated on-line using 100 mM H2SO4 delivered to the suppressor by a peristaltic pump at a flow rate of about 0.8 mL min-1. Separations were carried out using a Metrohm Metrosep A Supp 5 column (250 x 2 mm), and an eluent gradient composed by Na2CO3 and NaHCO3 was used at a constant ratio of 3.2:1. The final operating conditions involved the use of two eluents composed by Na2CO3/NaHCO3 at concentrations of 1/0.31 mM (A) and 10/3.1 mM (B), respectively and as per the manufacturer's recommendations. High pressure pumps were programmed at 100 % A for 19 min, which was later decreased at 0 % for 26 min using a step gradient. Re-equilibration at initial conditions was finally allowed for 20 min before the next injection. Injection loop was 125 µL, while column temperature was kept at 35 °C. Instrument control and data processing were performed on MagIC Net software version 3.1 from Metrohm.

2.3. HRMS conditions

HRMS experiments were carried out by coupling the Metrohm 850 Professional IC with an Exactive Orbitrap (Thermo Fisher Scientific, Sunnyvale, USA). Coupling was performed directly at the exit of the suppressed conductivity detector (SCD), thus providing serial SCD and HRMS detection in a single run. The Exactive Orbitrap system was equipped with a Thermo HESI-II source which allowed performing heated electrospray ionisation and no organic solvent eluate modification was employed. The source was used in negative ionisation mode. Full scan mode was used to acquire data over a 50 – 600 m/z range. Instrument resolution was set to 100,000 FWHM. The electrospray sheath gas flow and auxiliary gas flow rates were set to 60 and 10 arbitrary units, respectively. Spray voltage was 3 kV. The capillary temperature and voltage were 310 °C and -25 kV. The tube lens and skimmer voltages were -50 V and -18 V, whilst the HESI-II heater temperature was 350 °C. All data acquisition was performed using XCalibur software version 2.2 from Thermo Fisher Scientific.

2.4. Solid phase extraction

Four different co-polymeric mixed-mode SPE cartridges were tested in this work. These were Isolute ENV+ from International Sorbent Technology (Cambridge, UK), Oasis HLB from Waters (Milford, USA), LiChrolut EN from Merck (London, UK), and HyperSep Retain PEP from Thermo Fisher Scientific (Sunnyvale, USA). An anion-exchange resin was also tested at the outset of the work using Bond Elut Plexa PAX from Agilent Technologies (Santa Clara, USA) but disregarded as recoveries were low (data not shown) and to minimise potential extraction interference/sorbent breakthrough resulting from higher concentrations of inorganic anions such as chloride, sulphate and nitrate typically present in municipal waters.

A similar extraction protocol was generally adopted for all the selected cartridges, following the respective manufacturer's suggestions and previous work [18]. This involved pre-conditioning of cartridges by the successive use of 6 mL of methanol and 3 mL of Milli-Q water, followed by the loading of 100 mL of samples and a washing step of 1 mL of Milli-Q water. Recovery of extracted compounds from sorbent phases was finally accomplished with 3 mL of a 10 mM NaOH solution, after a drying time of 30 min. Before SPE, samples were acidified with 130 μL of concentrated H2SO4. For the selection of the best SPE cartridge, a mixture of 250 μg L-1 of the 10 reference HAAs in tap water was extracted. Percent recoveries were determined by quantifying the HAA concentrations in the injected extract though a calibration curve and comparing these values to those expected in case of 100 % recovery. The final method involved Isolute ENV+ as the SPE cartridge (see Results and discussion).

2.5. Performance evaluation

Selectivity, precision (as retention time and peak area repeatability), linear range, limits of detection (LODs) and limits of quantification (LOQs) of the entire procedure (i.e. including the SPE pre-concentration step) were assessed by spiking the 10 reference HAAs into drinking water sampled from our mains supply in Central London. For the most part, these experiments were conducted in line with the method validation guidelines proposed by the USEPA given the application area [19]. However, as little/no detector noise existed using HRMS in most cases, guidelines proposed by the International Council for Harmonisation (ICH) were used for LODs and LOQ estimation [20].

Repeatability was measured by the relative standard deviation (%RSD) of chromatographic retention time and peak areas, estimated by the successive analysis of replicated samples (n = 6) spiked at two concentrations of 10 μg L-1 and 100 μg L-1. Determination ranges were studied on samples spiked at 11 different concentration levels between 1 and 1000 μg L-1, which were analysed in duplicate. Both linear and quadratic calibration models were applied on data points, and lines of best fit allowing for coefficients of determination ($R2) \geq 0.99$ over the largest range were accepted. Finally, LODs and LOQs were estimated from the linear calibration curves of the different HAAs, and were respectively defined as 3 and 10 times the estimated standard deviations of peak areas divided by the slopes of the respective calibration curves.

2.6. Screening and identification of compounds

A grab sample (400 mL) of municipal drinking water was collected and divided into four separate aliquots, each of which was analysed with the developed method in parallel. Alongside providing high quality identification of targeted analytes, this was also considered especially important to demonstrate proof-of-concept of the in silico preliminary identification approach for new/additional compounds which could be supported by replicated IC-HRMS data for separately processed samples. Acquired full scan IC-HRMS chromatograms were analysed in two steps in order to screen for different compounds. At first, targeted analysis was carried out to determine the 10 pre-selected HAAs (i.e. those which reference materials existed). Then, the data was screened for 15 additional halogenated carboxylic acids not included in the method optimisation/validation exercise, i.e. monofluoroacetic acid (MFAA), difluoroacetic acid (DFAA), tribromoacetic acid (TBAA), monoiodoacetic acid (MIAA), diiodoacetic acid (DIAA), chloroiodoacetic acid (CIAA), bromoiodoacetic acid (BIAA), monochloropropionic acid (MCPA), dichloropropionic acid (DCPA), monobromopropionic acid (MBPA), dibromopropionic acid (DBPA), monochlorobutyric acid (MCBA), dichlorobutyric acid (DCBA), monobromobutyric acid (MBBA) and dibromobutyric acid

(DBBA). The list of untargeted analytes was intentionally composed of contaminants which have already been reported in previous works (as a benchmark) and others which have not been studied before (i.e., DFAA, MCPA, MBPA, DBPA, MBBA, DBBA). These compounds were therefore chosen in order to comprehensively evaluate the validity of the approach against reported data and novel applications.

In both targeted and suspect screening analyses, three characteristic ions were initially monitored for each compound: [M – H]–, [M – COOH]– and [2M – H]–. The most intense ion was selected, and its isotope pattern studied for preliminary identification, following the suggestions of Mol et al. [21]. To this end, preliminary identifications were retained if the observed accurate m/z for each isotope, as well as their relative isotopic abundances (RIAs), matched their theoretical values. For accurate m/z, a match was accepted if inaccuracies were < 5 ppm for all isotopes in the pattern of the investigated compound [22]. For RIAs, on the other hand, TraceFinder software version 3.1 (Thermo Fisher Scientific) was used to automatically compare observed and theoretical values, and finally measure an agreement probability (%ID). A match was accepted if %ID > 95 %. For the sake of comparison, manually determined values of RIAs were also determined on the basis of the isotope peak areas. Software generated RIA values were obtained using the Xcalibur internal isotope calculator (Thermo Fisher Scientific). Identifications of the retained peaks were finally confirmed via retention time for the targeted HAAs.

3. Results and discussion

3.1. Optimisation of IC conditions

The first stage of this work involved optimisation of IC gradient conditions, which was carried out using 10 reference HAAs showing different interaction strength with anion-exchange resins. A range of potentially suitable IC columns were briefly considered (data not shown) and a Metrosep A Supp 5 (250 x 2 mm) analytical column was ultimately chosen. This is an anion-exchange column packed with 5.0 µm particles of a quaternary ammonium-functionalised polyvinyl alcohol polymer. Its efficiency was empirically investigated on the reference HAAs and showed to be suitable for multi-residue analysis. A relatively flat van Deemter C term contribution at higher flow rates was observed (Figure S1 in Electronic Supplementary Material), which allowed for a slightly elevated flow rate of 0.21 mL min-1 to be used to minimise overall runtimes (the manufacturer's recommendation for general application was 0.18 mL min-1).

Gradient elution conditions were optimised using an eluent ratio between Na2CO3 and NaHCO3 of 3.2:1. The best separation between analytes was optimised in order to maximise peak purity, and

allow the best conditions for suspect-screening and characterisation of new/additional contaminants. Initial efforts to alter Na2CO3/NaHCO3 ratio showed no significant improvements in separation selectivity or baseline noise and were not further pursued. Good separation between the first seven eluting HAAs (MCAA, MBAA, TFAA, DCAA, BCAA, CDFAA, and DBAA) was reached with an isocratic 1/0.31 mM Na2CO3/NaHCO3 eluent. The last three eluting compounds (TCAA, BDCAA and CDBAA) required a gradient from 1/0.31 mM to 10/3.1 mM Na2CO3/NaHCO3 which was started after elution of DBAA. Overall, whilst longer than ideal, acceptable resolution for selected HAAs was achieved on the selected IC resin under these conditions over about 50 min (Fig. 1a). A shorter runtime would sacrifice resolution and was not considered optimal for suspect screening applications. Peak widths at half height ranged from 0.5 min (MBAA) to 1.15 min (CDBAA), with more tailing on later eluting compounds as perhaps expected.

Increased injection volume was investigated as a more convenient means to potentially achieve higher HRMS sensitivity and precision than via organic solvent addition either in the eluent or introduced post-suppressor [7, 15]. No significant reduction in peak shape or response linearity using three loop volumes (40, 86 and 125 µL) was observed. An injection loop volume of 125 µL was therefore selected.

3.2. Coupling IC to HRMS

In order to assure simplicity, direct coupling of HRMS to IC was the preferred option, with the suppressed aqueous eluate configured directly into the HESI source (which was heated). To this end, HESI conditions were optimised by direct source infusion of the 10 targeted HAAs in negative ion mode.

After coupling, HRMS spectra of targeted compounds were studied on repeated injections of a standard solution. HAAs have previously been shown to dimerise and/or fragment in ESI/HESI sources [7, 8] and this trend was also observed here using an Orbitrap mass analyser. Particularly, in addition to the pseudo-molecular ion [M – H]–, two other distinct ions were observed for most compounds, i.e. the decarboxylated fragment ion [M – COOH]– and the dimer ion [2M – H]–. Spectra of chlorinated and brominated compounds were furthermore characterised by the distinct presence of multiple intense isotopes for each fragment/adduct. Figure 2 shows examples of the observed full-scan spectra for DCAA and BCAA where these complex natural isotope patterns are evident, especially for dimer species.

For each fragment/adduct, the most abundant isotope was selected and m/z inaccuracies were studied (Table 1). Mean m/z inaccuracies below 2 ppm were obtained below 2 ppm for the majority of ions.

The only exception was the [M – COOH]– ion of MBAA, for which a mean inaccuracy of 4.42 ppm was observed, but which was still within the recognised threshold of 5 ppm [23]. The evaluation was extended to the other major isotopes in the respective isotope patterns and no significantly different results in measured m/z inaccuracies were observed. The use of carbonate/bicarbonate eluents did not result in any detectable interference in acquired HRMS spectra. Intra-day repeatability on measurements were very good with no significant increases in measured errors and/or trends on m/z values.

Relative abundances between the different fragments/adducts in the HRMS spectra of each HAAs were measured (Table 1). The latter displayed trends which depended on the degree and type of halogenation, as reported previously [7]. Particularly, stability of the [M – H]– ion was better for those HAAs substituted with a small number of light halogens such as MCAA, MBAA and CDFAA. Abundance of the [M – COOH]– ion increased for increasing degrees of substitution, and it became dominant for TCAA, BDCAA and CDBAA. Relative abundance of the [2M – H]– ion was generally low for each HAA under selected HESI conditions. No evidence of co-dimerisation between different partially eluting HAAs was observed.

Overall, this approach was considered especially suited to the suspect screening and preliminary in silico characterisation of additional compounds. Particularly, the presence of more discernible natural isotopes in HRMS spectra is valuable, as their ratios can be potentially exploited in addition to accurate m/z for identification of chlorinated and brominated analytes through comparison with expected natural distributions [21]. Relative abundances between ions formed either through unintended in-source collision-induced dissociation (CID, as here) or higher-energy collisional dissociation (HCD) could also potentially be used if needed for retrospective preliminary characterisation, and both operated in full-scan mode [21].

3.3. Selection of SPE sorbent

Despite the high selectivity provided by HRMS, the particular instrument used in this research was not sensitive enough to allow low-sub µg L-1 determinations directly in drinking water, even with relatively high injection volumes. Hence, it was decided to retain a SPE step using a co-polymeric mixed-mode sorbent in order to concentrate organic analytes in particular and potentially reduce any eventual interference on separation/ionisation from larger concentrations of inorganic ions normally present in drinking water.

Following acidification of samples to increase non-polar interactions, four relatively hydrophobic sorbents (i.e. LiChrolut EN, HyperSep Retain PEP, Oasis HLB and Isolute ENV+) were compared

with respect to recovery of the 10 selected HAAs (Fig. 3). As can be observed, LiChrolut EN (ethylvinylbenzene-divinylbenzene copolymer) and HyperSep Retain PEP (urea-functionalised styrene-divinylbenzene copolymer) showed the poorest retention performances with median recoveries amongst the targeted HAAs of 30 and 15 %, respectively. Both were unable to recover any quantifiable amount of CDBAA, with LiChrolut EN additionally not allowing extraction of CDFAA. Oasis HLB (divinylbenzene-N-vinylpyrollidone copolymer) presented better overall results, with a median recovery of 50 %. However, this sorbent could not retain quantifiable amounts of CDBAA, in addition to further limitations in extracting BDCAA. Isolute ENV+ (hydroxylated styrene-divinylbenzene copolymer) seemed to provide the best extraction performances. Median recovery for the selected HAAs was higher at 75 %. Furthermore, it was the only sorbent allowing the extraction of quantifiable amounts for all compounds, with overall better individual recoveries in comparison to all the other sorbents (except for CDFAA and TCAA, for which Oasis HLB performed better).

Results for LiChrolut EN were somewhat unexpected and were inconsistent with some previous works which showed better recoveries for these HAAs [18, 24]. Differences in sample processing existed however (including the initial sample volume and elution conditions) and inconsistencies were partially attributed to these. On the contrary, results for Oasis HLB were more consistent with previous research and those of Isolute ENV+ even showed improved performances with the simplified elution conditions used in this work [24]. The endpoint was not just to recover the 10 selected HAAs, but mainly to develop a flexible method for other ionic organic contaminants in drinking water. A generally applicable sorbent was thus preferred and Isolute ENV+ was finally chosen due to its better recovery for a larger range of compounds on the whole. The inclusion of a hydroxylated phenyl ring component to this polymer sorbent also likely promoted hydrogen bonding and other polar interactions, which was a distinct extra advantage over, say, LiChrolut EN.

3.4. Analytical method performance

In order to obtain a general idea of the analytical performance of the developed method, a quantifier ion was selected for each of the selected HAAs (i.e. the most intense fragment ion, Table 1) and figures of merit for the complete methodology, including the SPE step, were determined in matrix.

As can be seen from Figure 1a, IC-HRMS selectivity for the selected HAAs was excellent with a single chromatographic peak detected for each quantifier ion. Comparatively, IC-MS methods implementing low resolution mass analysers are unlikely to yield such selectivity across the full run time in the presence of matrix, even in SIM mode [7]. The main issue in this regard is the existence of isobaric species (i.e. species with same nominal m/z values, but different molecular formula)

which, if co-eluting, would be indistinguishable and thus interfere each other. A good example of this is demonstrated by the [M – H]– ion of MCAA (m/z = 92.9749) and [M – COOH]– ion of MBAA (m/z = 92.9341). Chromatographic peak extremities for these ions slightly co-eluted, but were still differentiable thanks complete resolution of their respective m/z signals using the HRMS parameters here, as shown in Figure 4. This arguably also represented better selectivity than is often achieved with triple quadrupole MS instruments working in SRM mode, where common fragment ions may exist for several compounds [9]. Practical benefits to data mining are well depicted in Figure 5, which compares EICs allowed by the developed IC-HRMS method to those which are usually obtained with most low resolution IC-MS methods in the retrospective screening of two non-targeted compounds. It can thus be observed that preliminary screening would not be possible in this case (and for most ions of interest) at low resolution, since co-eluting isobaric ions would mask signals of interest in background noise.

All numeric method performance data are given in Table 2. Peak area repeatability for all 10 HAAs in term of relative standard deviation (%RSD) were < 17 % and < 12 % after SPE of drinking water samples spiked at 10 µg L-1 and 100 µg L-1, respectively. These results were considered largely satisfactory considering that repeatability was measured in the actual matrix and after SPE. Retention time repeatability was also excellent at < 1.5 %RSD for all HAAs. Mean inaccuracy on measured m/z values of the quantifier ions in both extracts were comparable to those observed in standard solutions, demonstrating little/no influence of the SPE procedure or matrix on IC-HRMS measurements. Method linearity in matrix for targeted HAAs was acceptable, with values of R2 above 0.99 observed in all cases. Linearity was described either by linear or quadratic least-squares regression equations. In general, a quadratic equation described the linearity best over wider concentration ranges for 6 out 10 compounds (i.e. TFAA, DCAA, CDFAA, TCAA, BDCAA and CDBAA), with the two fluorinated HAAs especially benefitting from the adoption of a quadratic calibration equation. These results were not surprising, since curvature in HRMS calibrations has been shown to occur frequently in other applications using Orbitrap-type instruments [22, 25, 26]. Using the optimal calibration models, upper limits for determination ranges were of 500 µg L-1 for TFAA and DCAA, 750 µg L-1 for CDFAA and TCAA and 1000 µg L-1 for the other targeted compounds.

Little or no noise was observed for most compounds, as is often the case when working with EICs with such HRMS instruments. Despite this apparently high sensitivity, according to the ICH definition LODs were estimated between 0.46 to 2.30 µg L-1, with MCAA and MBAA showing the best sensitivity (0.51 and 0.46 µg L-1, respectively) and TFAA the poorest (2.30 µg L-1). Recent

works using direct injection (DI) and IC coupled to tandem MS analysers offered better LODs in general than here [9, 10]. However, direct comparison with other methods where LODs were estimated using different criteria, e.g. a 3:1 signal-to-noise ratio threshold, arguably should be considered with care as well as whether LODs have been measured in matrix or not. Here, we opted for simplicity of the IC-HRMS configuration. However, the addition of organic solvent to the eluent [15] or added post-suppressor [7] has previously been shown to improve sensitivity by approximately half an order of magnitude and could consequently be tested in future works. It is worth noting too that other HRMS instruments currently available (including those incorporating Orbitrap mass analysers, e.g. the Exactive Plus or QExactive Plus) now offer higher sensitivity compared to that used in this contribution and may thus allow to further improve performance, as well as potentially negate the SPE step.

Overall, the approach adopted here was still deemed appropriate for the purpose of the current qualitative and quantitative applications to halogenated organic acids, including in silico suspect screening for new compounds.

3.5. Targeted analysis of reference HAAs

The method was applied to the determination of the 10 HAAs in an unspiked drinking-water sample through the analysis of acquired full-scan IC-HRMS chromatograms. As this work focused mainly on the potential benefits of IC-HRMS for screening and identification of compounds of interest in single samples, an extended occurrence study was not performed. Such reports can be found elsewhere (e.g. [27-30]).

The screened sample consisted in a grab sample of municipal drinking water, which was collected and divided in four aliquots. Each aliquot was separately processed and analysed. As perhaps expected, no occurrence of any targeted HAAs was detected in suppressed conductivity traces, even with an SPE step. However, relatively high concentrations of chloride, nitrate and sulphate were still detected at retention times of 14.64, 23.47 and 39.95 min, respectively, despite little/no selectivity of the SPE sorbent for them. These inorganic ions partially co-eluted with some HAAs, and thus could prevent their detection and reliable determination in conductivity chromatograms.

Full-scan HRMS chromatograms were analysed, and the ions characteristics of the 10 HAAs were extracted (i.e. [M – H]–, [M – COOH]– and [2M – H]–). Contrary to conductivity chromatograms, EICs were not affected by co-extracted contaminants and actually revealed very distinct HAA peaks. Particularly, each analyte presented one single chromatographic peak at the retention time of the respective most intense ions previously identified (Fig. 1b). For most analytes (all except MCAA,

MBAA and CDFAA), additionally co-eluting peaks were also observed in the EICs for the other fragments/adducts composing the respective in-source CID fragmentation patterns. Relative abundances were calculated and were largely comparable to those previously observed on standard solutions during method development, providing preliminary evidence for the presence of the 10 HAAs in the sample.

Identification of detected peaks was confirmed via retention time, accurate m/z values and isotopic distributions. Relative isotope abundances (RIAs) were particularly examined for ions which were likely to belong to chlorinated and brominated compounds, while TraceFinder software was used to quantitatively measure the probability of match between observed and expected values (%IDs). Results are reported in Table 3. It was thus observed that inaccuracies in m/z measurements were generally < 3 ppm for all the monitored isotopes. Measured RIAs largely met those expected in nature, with differences usually < 7 %. Exceptions were CDFAA, BDCAA and CDBAA, for which differences between observed and expected RIAs were significantly higher for some isotopes. This is, however, not surprising as differences between RIAs has been shown to occur using Orbitrap-type instruments, especially at such low signal intensity and/or for ions with rich isotope fine structures [21, 31]; characteristics that were both met in this case by CDFAA, BDCAA and CDBAA. Automatic comparison of isotopic distributions through TraceFinder did not actually detect any inexplicable incompatibility, and resulting %IDs were overall > 95 % (see Figure 6a for an example of BDCAA). Observed differences between measured and theoretical RIAs were thus deemed not significant, and the presence of all the 10 targeted HAAs in the sample was finally confirmed though matching retention times with standards. Blanks analysed before and after sample analyses were clean. Little or no carryover existed in sequential runs of high concentration range standards and blanks.

The concentrations of the 10 HAAs in the sample were then determined by standard addition (Table 3). These were generally low, with three compounds falling below the LOQ and those remaining having concentrations ranging from 2.59 μg L-1 to 8.16 μg L-1 (for MCAA and DBAA, respectively). Traces of DCAA were detected, which complied with the WHO guidelines, but not fully with the goals set by the USEPA. The concentrations of all other HAAs were within the limits suggested by the different regulatory bodies. All concentrations determined were consistent with ranges reported in an extensive 2011 survey of UK waters for HAA content including DCAA and TCAA [27].

3.6. Suspect screening of additional compounds

A main benefit of using HRMS detection lies in the possibility of retrospectively analysing acquired data in order to more rapidly identify new or emerging compounds. To assess this potential

application, the data acquired from the previous four samples were screened for 15 additional carboxylic acids not included in the method optimisation/validation exercise (see Table 4 for the full compound list). The latter encompassed iodinated HAAs which were recently reported to be a potentially new (but still not regulated) class of DBPs in drinking water [32, 33], as well as halogenated propionic and butyric acids such as Dalapon (i.e., 2,2-dichloropropanoic acid).

As with targeted HAAs, three ions were initially monitored for each compound: [M – H]–, [M – COOH]– and [2M – H]–. No chromatographic peaks in the corresponding EICs were detected at all for four suspects (i.e. DIAA, MFAA, MBBA and DBBA). Of those remaining, each compound presented at least one peak in the corresponding EICs which were detected in all four replicates, while 3 of them showed more than one peak at different retention times (i.e. MCPA, MBPA and BIAA). In the case of MCPA and MBPA, these could be explained by different structural isomers, but for BIAA such structural isomers cannot exist. Overall, a total of 14 peaks were detected. Most of these had very low intensity with peak areas < 1000 counts and signal-to-noise ratios < 10. Only 6 compounds showed higher intensities, i.e. the two peaks preliminarily attributed to MCPA, the late eluting peak preliminarily attributed to BIAA, and those preliminarily attributed to MIAA, CIAA, and DCPA.

Preliminary in silico identification of the 14 chromatographic peaks detected was pursued via their accurate m/z values and isotopic distributions as before (Table 4). Inaccuracies of m/z measurements of the peaks were again generally < 3 ppm for all the monitored isotopes in comparison to calculated exact m/z. Furthermore, the investigated ions qualitatively presented all the expected natural isotopes for ions matching their presumed identities. The only exceptions were the late eluting peak preliminarily attributed to BIAA and that preliminarily attributed to MBPA, of which HRMS spectra actually showed the presence of the 79Br isotope but lacked any contributing 81Br signals. At this stage, this indicated negative preliminary identifications.

For a more quantitative approach to suspect identification, RIAs could be examined. As previously, however, some RIAs presented discrepancies with their respective theoretical values, which can eventually be accounted for by the low signal intensity and rich isotope fine structures of the related ions. TraceFinder was again used to effectively quantify match probabilities between isotope distributions. A %ID of 0 % was thus observed for the late eluting peak preliminarily attributed to BIAA, confirming previous inconsistencies in its qualitative isotopic distribution and negative identification. Because of their low intensities, no reliable results were furthermore obtained for the 8 lower intensity peaks. The remaining 5 peaks returned %IDs of > 95 % and would thus be preliminary identified as their corresponding suspected ions (i.e. MIAA, CIAA, DCPA and MCPA).

For the sake of illustration, Figure 6b shows the results of the automatic comparison between the observed and theoretical isotope distributions of one of the screened compounds (i.e., MCPA).

Amongst the preliminary identified peaks, it was interesting to note that both peaks detected in the EICs of the [M – H]– ion of MCPA actually matched the expected isotope distributions and m/z values. As previously presumed, this result might indicate two different structural isomers (i.e. 2-MCPA and 3-MCPA). The peak matching the [M – H]– isotope pattern of DCPA could be due to Dalapon (2,2-DCPA), a herbicide frequently encountered as a contaminant in drinking water [34]. Preliminary identities should eventually be confirmed by the injection of reference standards. Given the unavailability of the latter at the time of analysis, this confirmation procedure was unfortunately not undertaken. However, a comparison with the literature revealed that the relative elution order of MIAA, CIAA and 2,2-DCPA to the 10 targeted HAAs on similar anion-exchange resins is fully consistent with that observed here [9, 10]. No data on the separation and elution order of MCPA isomers in IC have been found, and this makes the current work the first to report their presumptive presence in drinking water, entirely using in silico IC-HRMS data mining.

4. Conclusion

In this work, a novel IC-HRMS method was developed for the screening of halogenated carboxylic acids in drinking water. In particular, of four SPE cartridges tested, Isolute ENV+ showed the best overall recoveries for 10 selected HAAs. Analytical method performance was acceptable for screening applications, as well as for quantitative determinations. LODs were in the µg L-1 concentration range making it fit for purpose with excellent selectivity, repeatability and linearity.

The method was applied to four independent samples from the same municipal drinking-water source. Targeted analysis successfully identified and quantified all 10 selected HAAs in all samples, even if not present at hazardous concentrations. Furthermore, 4 new compounds were preliminarily identified in silico in suspect screening mode upon consideration of their accurate m/z values, isotope patterns and expected elution orders. These included 2 iodinated HAAs (i.e. monoiodoacetic acid and chloroiodoacetic acid) and 2 chlorinated propionic acids (i.e. monochloropropionic acid and dichloropropionic acid, also known as Dalapon). Both isomers of monochloropropionic acid were separated by IC and potentially shown to be present in drinking water for the first time.

The developed method was therefore flexible for both targeted analysis and suspect screening. Importantly, potential to retrospectively investigate acquired IC-HRMS chromatograms for new or emerging water contaminants has been proven. This approach could be extended further to screen for other DBPs or compounds of special interest in drinking waters which may be present at low-sub µg

L-1 concentrations, and to ultimately perform extended occurrence studies. Future research using IC-HRMS in this field is thus very promising.

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Table 1 – Observed inaccuracy in m/z values ($\delta_{m/z}$, n = 9) and relative intensity (RI, n = 5) for different ions related to 10 reference HAAs, measured through repeated injection of an aqueous standard solution (1 mg L^{-1}). Values in bold are the quantifier ions selected for method performance evaluation.

HAA	***[M – H]⁻***			***[M – COOH]⁻***			***[2M – H]⁻***		
	Exact m/z[a]	***$\delta_{m/z}$ (ppm)***	***RI (%)***	***Exact m/z[a]***	***$\delta_{m/z}$ (ppm)***	***RI (%)***	***Exact m/z[a]***	***$\delta_{m/z}$ (ppm)***	***RI (%)***
MCAA	**92.9749**	-0.48 ± 0.78	100.00 ± 0.00	N/A	N/A	N/A	186.9570	-0.71 ± 0.71	0.96 ± 0.06
MBAA	**136.9244**	-0.49 ± 0.73	100.00 ± 0.00	92.9345	4.42 ± 1.74	0.01 ± 0.01	276.8540	0.24 ± 0.70	2.31 ± 0.12
TFAA	**112.9856**	-0.79 ± 0.53	100.00 ± 0.00	68.9958	0.81 ± 0.76	18.44 ± 1.23	226.9785	-0.24 ± 0.39	4.21 ± 0.39
DCAA	**126.9359**	-1.05 ± 0.56	100.00 ± 0.00	82.9461	-0.54 ± 0.64	27.87 ± 1.49	256.8761	-0.91 ± 0.55	1.92 ± 0.12
BCAA	**170.8854**	-1.37 ± 0.59	100.00 ± 0.00	126.8956	-0.61 ± 0.53	20.24 ± 1.07	344.7760	0.26 ± 0.61	2.47 ± 0.11
CDFAA	**128.9560**	-1.38 ± 0.52	100.00 ± 0.00	84.9662	-0.52 ± 0.62	0.18 ± 0.01	258.9194	-0.09 ± 0.57	4.52 ± 0.22
DBAA	**216.8328**	-0.67 ± 0.57	100.00 ± 0.00	172.8430	-0.45 ± 0.63	36.63 ± 1.84	434.6729	0.00 ± 0.40	1.06 ± 0.12
TCAA	160.8969	-1.31 ± 0.49	26.24 ± 1.49	**116.9071**	-0.86 ± 0.6	100.00 ± 0.00	324.7982	-0.24 ± 0.53	1.07 ± 0.08
BDCAA	204.8464	-0.38 ± 0.59	35.14 ± 0.90	**160.8566**	-1.04 ± 0.76	100.00 ± 0.00	414.6951	-0.64 ± 0.89	0.38 ± 0.07
CDBAA	250.7939	-0.04 ± 0.61	5.32 ± 0.52	**206.8040**	-0.48 ± 0.64	100.00 ± 0.00	502.5950	1.19 ± 2.32	0.00 ± 0.00

[a] most abundant isotope.

Table 2 – Figures of merit (using the most intense ions) estimated on spiked drinking-water samples. Determination ranges were inspected using linear and quadratic models, and are reported as the intervals of concentrations allowing for $R^2 \geq 0.99$. M/z inaccuracy ($\delta_{m/z}$), as well as repeatability of retention time (t_R) and peak areas (PA), were measured (n = 6) in samples spiked at two different concentrations: 10 and 100 µg L^{-1}. All data (LOD and LOQ included) were measured on samples which were subject to SPE pre-concentration, and are thus representative of the entire analytical procedure.

HAA	***t_R*** [a]	***$\delta_{m/z}$ (ppm)***		***Repeatability t_R (%RSD)***		***Repeatability PA (%RSD)***		***LOD (µg L^{-1})***	***LOQ (µg L^{-1})***	***Determination ranges (upper limit) (µg L^{-1})***	
		@ 10 µg L^{-1}	***@ 100 µg L^{-1}***	***@ 10 µg L^{-1}***	***@ 100 µg L^{-1}***	***@ 10 µg L^{-1}***	***@ 100 µg L^{-1}***			***Linear***	***Quadratic***
MCAA	14.53 ± 0.05	-0.54 ± 0.59	-0.18 ± 0.44	0.62	0.32	8.4	7.4	0.51	1.70	1000	1000
MBAA	15.52 ± 0.05	-0.73 ± 0.46	-0.73 ± 0.00	0.62	0.34	10.9	9.0	0.46	1.53	1000	1000
TFAA	18.92 ± 0.03	-1.18 ± 0.72	-0.89 ± 0.00	0.58	0.17	9.7	7.3	2.30	7.67	50	500
DCAA	19.06 ± 0.03	-1.18 ± 0.43	-0.79 ± 0.00	1.38	0.15	9.1	11.3	1.28	4.28	250	500
BCAA	22.06 ± 0.05	-1.56 ± 0.30	-1.46 ± 0.32	1.32	0.24	7.2	7.1	1.19	3.98	1000	1000
CDFAA	25.17 ± 0.12	-1.42 ± 0.58	-1.55 ± 0.00	0.39	0.47	9.6	8.5	1.25	4.16	50	750
DBAA	26.34 ± 0.08	-0.85 ± 0.35	-0.77 ± 0.24	0.29	0.31	7.6	6.4	1.00	3.33	1000	1000
TCAA	37.37 ± 0.05	-1.28 ± 0.47	-1.14 ± 0.44	0.37	0.12	15.7	7.8	1.14	3.81	250	750
BDCAA	40.33 ± 0.05	-1.14 ± 0.47	-1.04 ± 0.32	0.32	0.11	16.8	8.8	1.21	4.02	250	1000
CDBAA	44.56 ± 0.05	-0.56 ± 0.48	-0.73 ± 0.26	0.31	0.12	16.4	7.4	0.84	2.80	250	1000

[a] values determined on the samples spiked at 100 µg L^{-1} (n = 6).

Table 3 – Results after targeted analysis of four independent samples from the same municipal drinking-water source (n = 4). The column "detected fragments/adducts" reports the measured relative intensity (RI) between the different observed fragments/adducts of each compound. The column "target ion", reports the data concerning the three most abundant isotopes for the respective target ion (marked in bold) sorted from the most to the least abundant. Isotope data included observed inaccuracy on m/z values ($\delta_{m/z}$), as well as theoretical and observed relative isotope abundances (RIAs). Match probabilities of the isotope patterns (%ID) and estimated concentrations are also reported.

HAA	***t_R (min)***	***Detected fragments/adducts***			***Target ion***									***%ID***	***[HAA] ($\mu g\ L^{-1}$)[a]***
		$[M-H]^-$	***$[M-COOH]^-$***	***$[2M-H]^-$***	***Most abundant isotope***			***2nd isotope***			***3th isotope***				
		RI (%)	***RI (%)***	***RI (%)***	***$\delta_{m/z}$ (ppm)***	***Theo. RIA (%)***	***Obs. RIA (%)***	***$\delta_{m/z}$ (ppm)***	***Theo. RIA (%)***	***Obs. RIA (%)***	***$\delta_{m/z}$ (ppm)***	***Theo. RIA (%)***	***Obs. RIA (%)***		
Monochloroacetic acid (MCAA)	14.39 ± 0.02	**100.00 ± 0.00**	N/A	n.d.	-1.08 ± 0.00	100.00 (M)	100.00 ± 0.00	-2.63 ± 0.61	31.96 (M + 2)	30.49 ± 0.35	N/A	N/A	N/A	100.00	2.59 ± 0.58
Monobromoacetic acid (MBAA)	15.42 ± 0.08	**100.00 ± 0.00**	n.d.	n.d.	-1.28 ± 0.37	100.00 (M)	99.96 ± 0.08	-1.62 ± 0.36	97.28 (M + 2)	98.96 ± 1.14	N/A	N/A	N/A	100.00	< LOQ
Trifluoroacetic acid (TFAA)	18.65 ± 0.04	**100.00 ± 0.00**	16.67 ± 0.70	0.02 ± 0.02	-1.77 ± 0.00	100.00 (M)	100.00 ± 0.00	N/A	N/A	N/A	N/A	N/A	N/A	100.00	< LOQ
Dichloroacetic acid (DCAA)	20.76 ± 0.05	**100.00 ± 0.00**	26.82 ± 0.21	0.01 ± 0.01	-1.58 ± 0.00	100.00 (M)	100.00 ± 0.00	-1.55 ± 0.00	63.92 (M + 2)	63.67 ± 0.33	-1.53 ± 0.00	10.22 (M + 4)	9.13 ± 0.12	100.00	5.49 ± 0.43
Bromochloroacetic acid (BCAA)	22.36 ± 0.30	**100.00 ± 0.00**	18.19 ± 0.17	n.d.	-1.76 ± 0.00	100.00 (M)	100.00 ± 0.00	-1.16 ± 0.00	97.28 (M + 2)	96.67 ± 0.73	-1.14 ± 0.00	31.09 (M + 4)	29.11 ± 0.40	97.50	7.20 ± 0.69
Chlorodifluoroacetic acid (CDFAA)	25.13 ± 0.11	**100.00 ± 0.00**	n.d.	n.d.	-1.94 ± 0.45	100.00 (M)	100.00 ± 0.00	-0.38 ± 0.44	31.96 (M + 2)	8.32 ± 5.83	N/A	N/A	N/A	95.90	< LOQ
Dibromoacetic acid (DBAA)	26.26 ± 0.05	**100.00 ± 0.00**	35.11 ± 0.23	n.d.	-1.15 ± 0.27	100.00 (M + 2)	100.00 ± 0.00	-1.40 ± 0.00	51.40 (M)	48.50 ± 0.62	-0.91 ± 0.00	48.64 (M + 4)	46.44 ± 0.99	100.00	8.16 ± 0.66
Trichloroacetic acid (TCAA)	37.51 ± 0.05	22.43 ± 0.52	**100.00 ± 0.00**	n.d.	-1.71 ± 0.00	100.00 (M)	100.00 ± 0.00	-0.84 ± 0.00	95.88 (M + 2)	95.51 ± 0.77	-1.65 ± 0.00	30.65 (M + 4)	26.98 ± 0.48	95.74	6.47 ± 0.75
Bromodichloroacetic acid (BDCAA)	40.57 ± 0.08	29.98 ± 0.88	**100.00 ± 0.00**	n.d.	-1.87 ± 0.00	100.00 (M)	100.00 ± 0.00	-1.84 ± 0.00	97.28 (M + 2)	84.78 ± 2.16	-1.82 ± 0.00	62.18 (M + 4)	55.34 ± 1.52	97.90	6.44 ± 0.72
Chlorodibromoacetic acid (CDBAA)	44.80 ± 0.06	1.13 ± 0.30	**100.00 ± 0.00**	n.d.	-0.97 ± 0.00	100.00 (M + 2)	100.00 ± 0.00	-0.98 ± 0.00	51.40 (M)	45.21 ± 0.53	0.12 ± 0.24	48.64 (M + 4)	57.26 ± 0.96	100.00	3.04 ± 0.32

n.d.: not detected.

[a] < LOQ = compound detected but concentration below its lower limit of quantitation.

Table 4 – Summary of suspect screening for 15 halogenated carboxylic acids in four independent samples from the same municipal drinking-water source (n = 4). Only one ion per compound was generally detected and the column "target ion" reports observed characteristics concerning the three most abundant isotopes of that ion, sorted from the most to the least abundant. These included observed inaccuracy on m/z values ($\delta_{m/z}$), as well as comparative theoretical and observed relative isotope abundances (RIAs). Match probabilities of the isotope patterns (%ID) are also reported and the underlined values indicates compounds of which characteristics acceptably matched those of their expected identities.

Suspected identity	***t_R (min)***	***Proposed ion***	***Exact m/z[a]***	***Target ion***									***%ID[b]***
				Most abundant isotope			***2nd Isotope***			***3th Isotope***			
				$\delta_{m/z}$ (ppm)	***Theo. RIA (%)***	***Obs. RIA (%)***	***$\delta_{m/z}$ (ppm)***	***Theo. RIA (%)***	***Obs. RIA (%)***	***$\delta_{m/z}$ (ppm)***	***Theo. RIA (%)***	***Obs. RIA (%)***	
Monofluoroacetic acid (MFAA)	n.d	n.d.	n.d.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Difluoroacetic acid (DFAA)	15.48 ± 0.21	$[M - H]^-$	94.9950 (M)	-1.58 ± 0.61	100.00 (M)	100.00 ± 0.00	N/A	N/A	N/A	N/A	N/A	N/A	< TOM
Tribromoacetic acid (TBAA)	51.00 ± 0.23	$[M - COOH]^-$	250.7535 (M + 2)	-1.22 ± 0.68	100.00 (M + 2)	100.00 ± 0.00	-1.48 ± 0.68	97.28 (M + 4)	65.05 ± 31.51	-0.39 ± 2.22	31.54 (M)	8.73 ± 1.36	< TOM
Monoiodoacetic acid (MIAA)	16.39 ± 0.12	$[M - H]^-$	184.9105 (M)	-1.22 ± 0.68	100.00 (M)	100.00 ± 0.00	N/A	N/A	N/A	N/A	N/A	N/A	**100.00**
Diiodoacetic acid (DIAA)	n.d	n.d.	n.d.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Chloroiodoacetic acid (CIAA)	26.45 ± 0.10	$[M - H]^-$	218.8715 (M)	-0.11 ± 0.23	100.00 (M)	100.00 ± 0.00	-0.91 ± 0.98	31.96 (M + 2)	3.76 ± 1.66	N/A	N/A	N/A	**97.90**
Bromoiodoacetic acid (BIAA)	26.24 ± 0.07	$[M - H]^-$	262.8210 (M)	-0.86 ± 0.36	100.00 (M)	72.80 ± 25.10	-0.38 ± 0.53	97.28 (M + 2)	94.73 ± 10.54	N/A	N/A	N/A	< TOM
	30.96 ± 0.17	$[M - COOH]^-$	218.8312 (M)	0.91 ± 0.00	100.00 (M)	100.00 ± 0.00	n.d.	97.28 (M + 2)	n.d.	N/A	N/A	N/A	0.00
Monochloropropionic acid (MCPA)	14.60 ± 0.05	$[M - H]^-$	106.9905 (M)	-1.87 ± 0.00	100.00 (M)	100.00 ± 0.00	-0.92 ± 0.00	31.96 (M + 2)	20.91 ± 2.56	N/A	N/A	N/A	**99.75**
	36.04 ± 0.04	$[M - H]^-$	106.9905 (M)	-1.87 ± 0.00	100.00 (M)	100.00 ± 0.00	-0.92 ± 0.00	31.96 (M)	10.96 ± 4.15	N/A	N/A	N/A	**96.80**
Dichloropropionic acid (DCPA)	21.31 ± 0.42	$[M - H]^-$	140.9516 (M)	-1.42 ± 0.00	100.00 (M)	100.00 ± 0.00	-1.40 ± 0.00	63.92 (M + 2)	52.53 ± 1.70	N/A	N/A	N/A	**99.07**
Monobromopropionic acid (MBPA)	22.91 ± ?[b]	$[M - H]^-$	150.9400 (M)	2.65[c]	100.00 (M)	100.00[c]	n.d.	97.28 (M + 2)	n.d.	N/A	N/A	N/A	< TOM
	36.35 ± 0.18	$[M - H]^-$	150.9400 (M)	-1.10 ± 1.01	100.00 (M)	88.11 ± 20.60	0.98 ± 3.24	97.28 (M + 2)	90.59 ± 13.31	N/A	N/A	N/A	< TOM
Dibromopropionic acid (DBPA)	30.20 ± 0.05	$[M - H]^-$	230.8485 (M + 2)	-0.65 ± 0.25	100.00 (M + 2)	100.00 ± 0.00	-0.33 ± 1.44	51.40 (M)	11.91 ± 5.16	-1.50 ± 0.89	48.64 (M + 4)	9.83 ± 4.03	< TOM

Monochlorobutyric acid (MCBA)	16.09 ± 0.10	$[M - H^-$	121.0062 (M)	-2.07 ± 0.58	100.00 (M)	100.00 ± 0.00	-2.44[c]	31.96 (M + 2)	75.80[c]	N/A	N/A	N/A	< TOM
Dichlorobutyric acid (DCBA)	28.38 ± 0.19	$[M - H]^-$	154.9672 (M)	-1.61 ± 0.83	100.00 (M)	100.00 ± 0.00	-1.70 ± 1.33	63.92 (M + 2)	31.28 ± 17.36	N/A	N/A	N/A	< TOM
Monobromobutyric acid (MBBA)	n.d	n.d.	n.d.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Dibromobutyric acid (DBBA)	n.d	n.d.	n.d.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

n.d.: not detected.

[a] most abundant isotope.

[b] < TOM = peak area and signal-to-noise ratio of the respective chromatographic peak below the pre-set thresholds of measurement for TraceFinder (1000 counts and 10, respectively).

[c] compound and/or isotope detected in only 1 of the 4 sample aliquots.

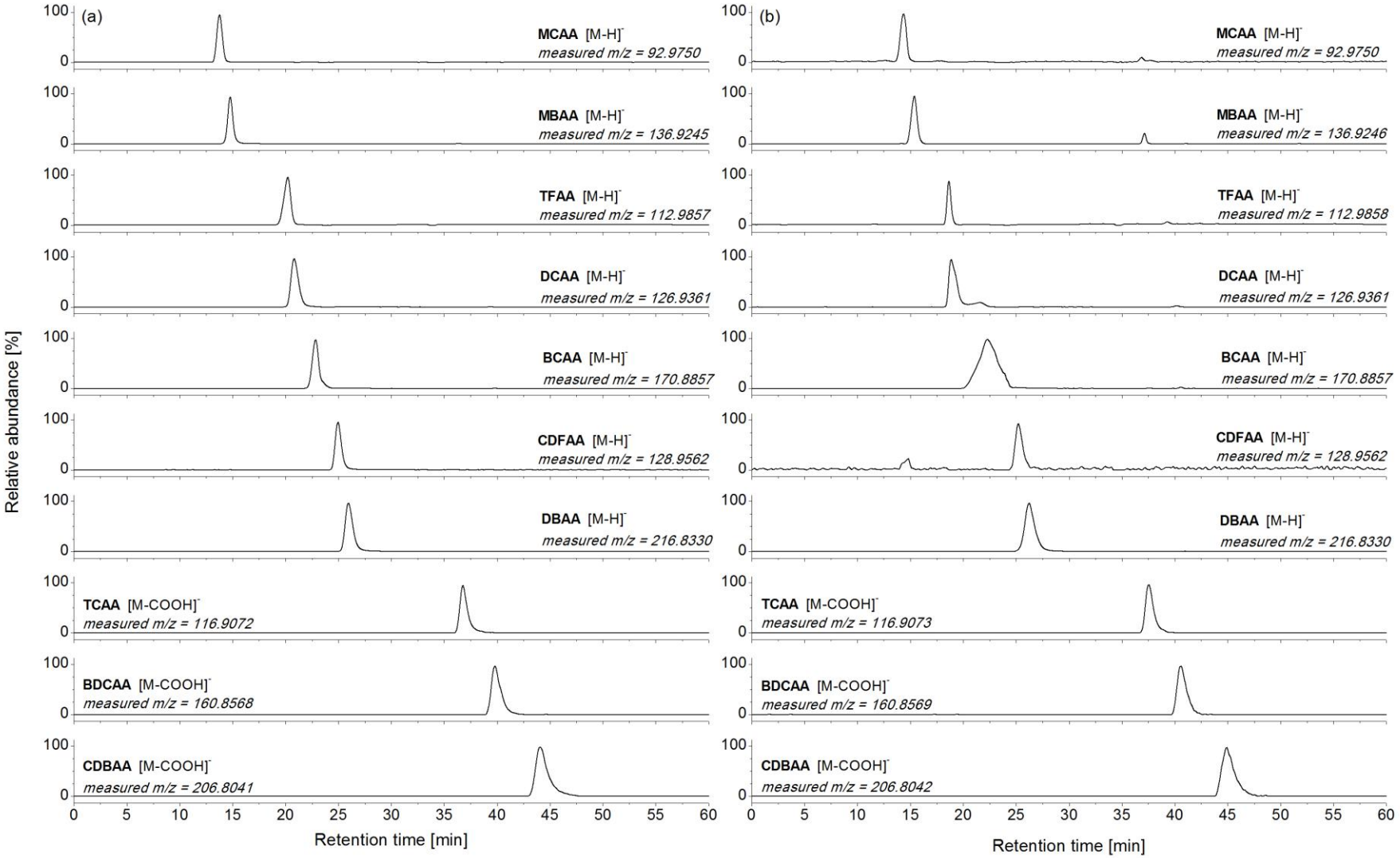

Figure 1 – Extracted-ion chromatograms (EICs) of a tap-water sample spiked at a concentration of 5 μg L^{-1} (a) and an unspiked drinking-water sample after analysis with the developed IC-HRMS method (b). In (b), the most intense fragment ions of all the 10 reference HAAs were detected.

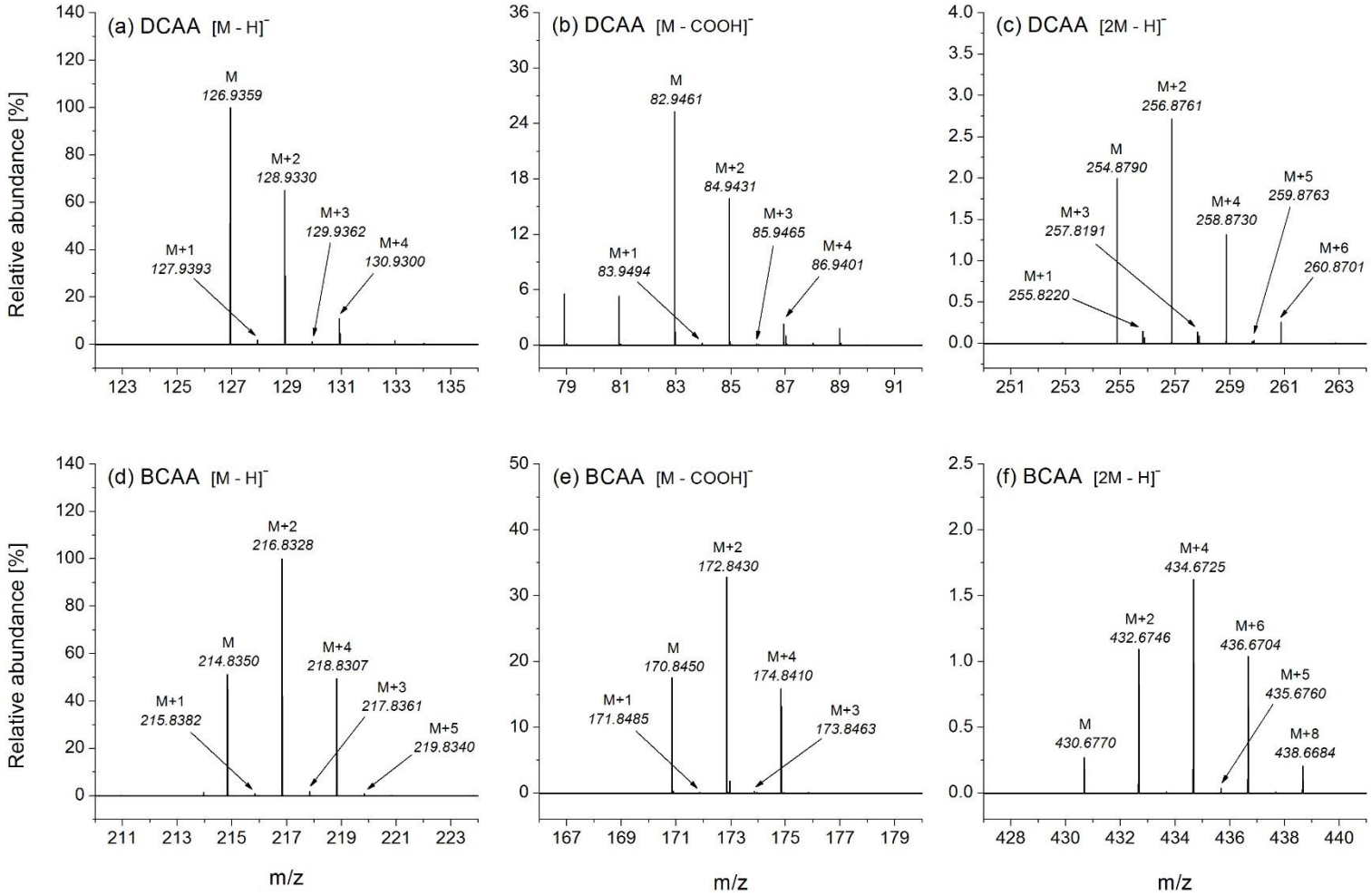

Figure 2 – Examples of isotope patterns for two reference HAAs (DCAA and BCAA) and their respective HESI fragments/adducts (i.e. ion $[M - H]^-$, $[M - COOH]^-$ and $[2M - H]^-$).

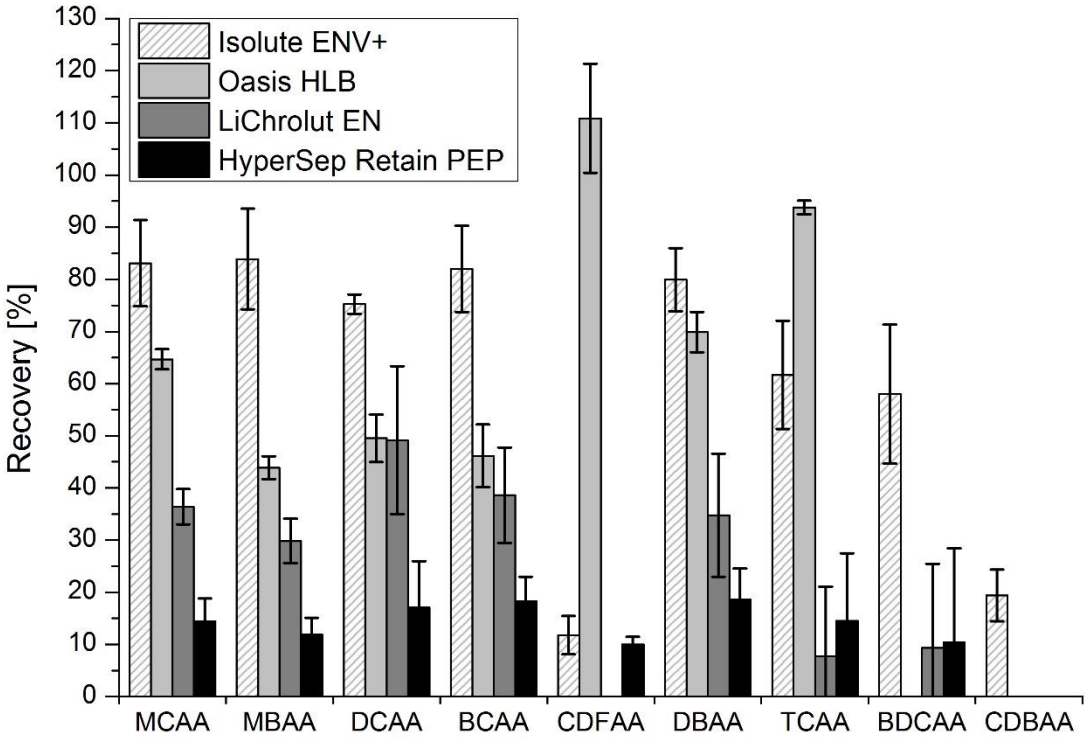

Figure 3 – Observed recoveries (n = 3) for reference HAAs after SPE using four different sorbents. Tests were performed using a spiked solution of 250 μg L^{-1} HAAs in drinking water.

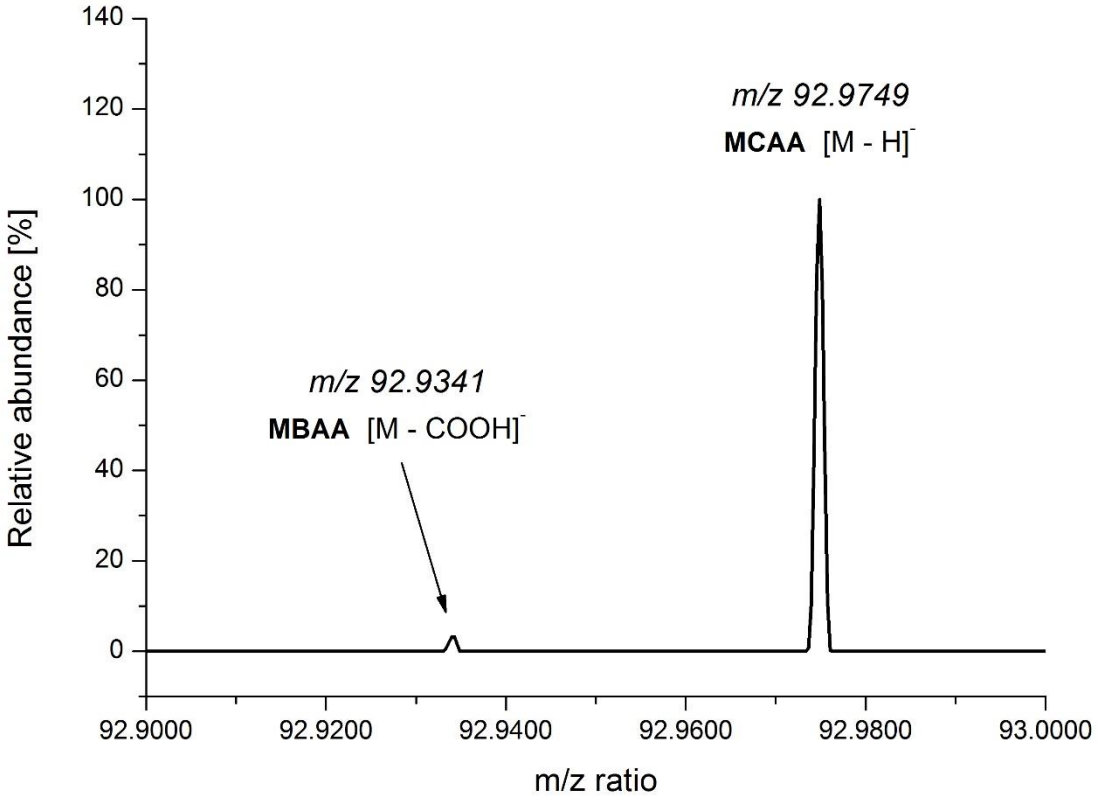

Figure 4 – HRMS spectrum representing the complete discrimination between two HAA-related compounds with very similar m/z values: the $[M - COOH]^-$ ion of MBAA (exact m/z = 92.9345) and the $[M - H]^-$ ion of MCAA (exact m/z = 92.9749).

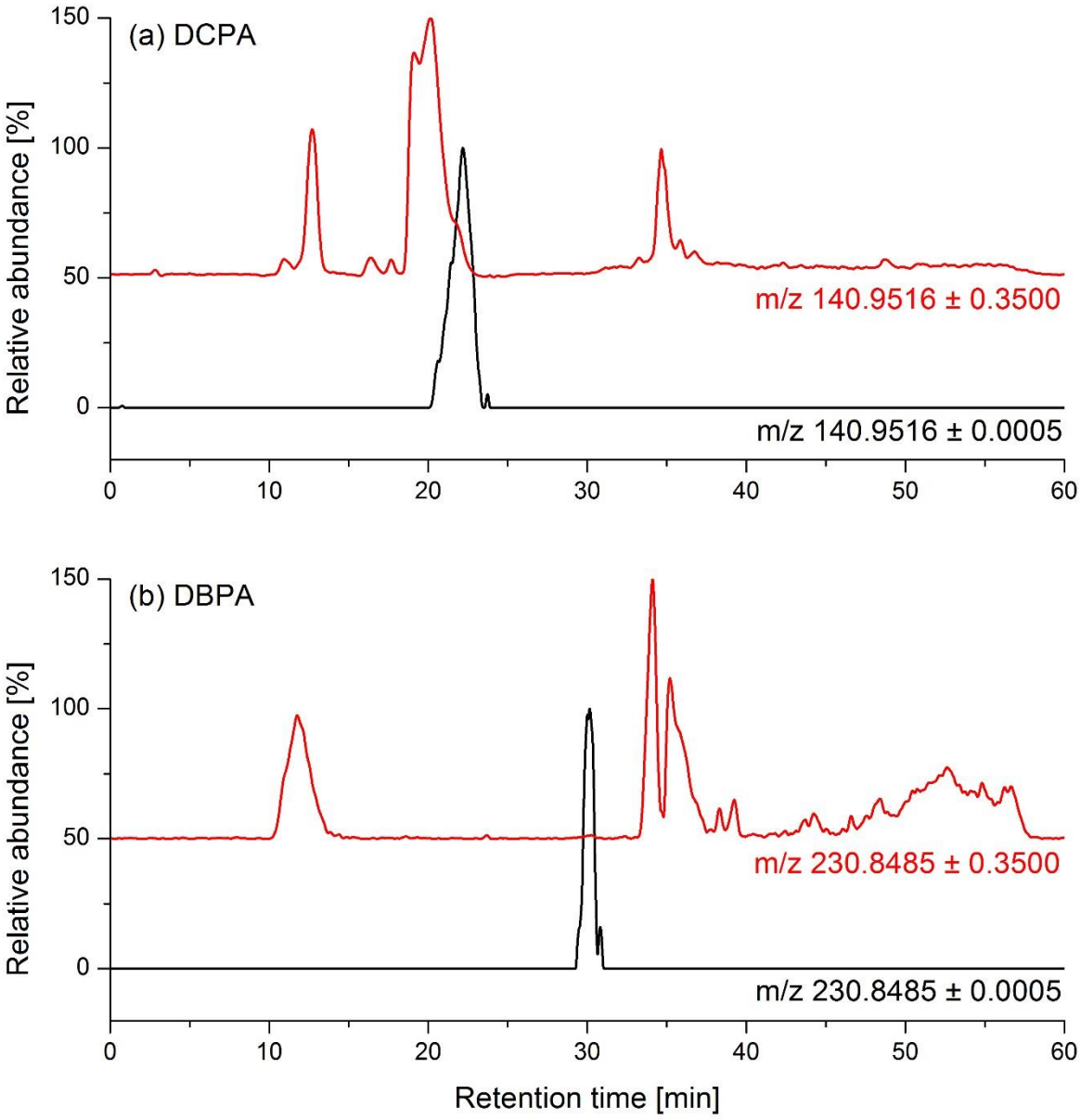

Figure 5 – Comparison between simulated low-resolution (red) and high-resolution (black) EICs for the suspect screening of two suspected compounds, (a) dichloropropanoic acid (DCPA) and (b) dibromopropanoic acid (DBPA).

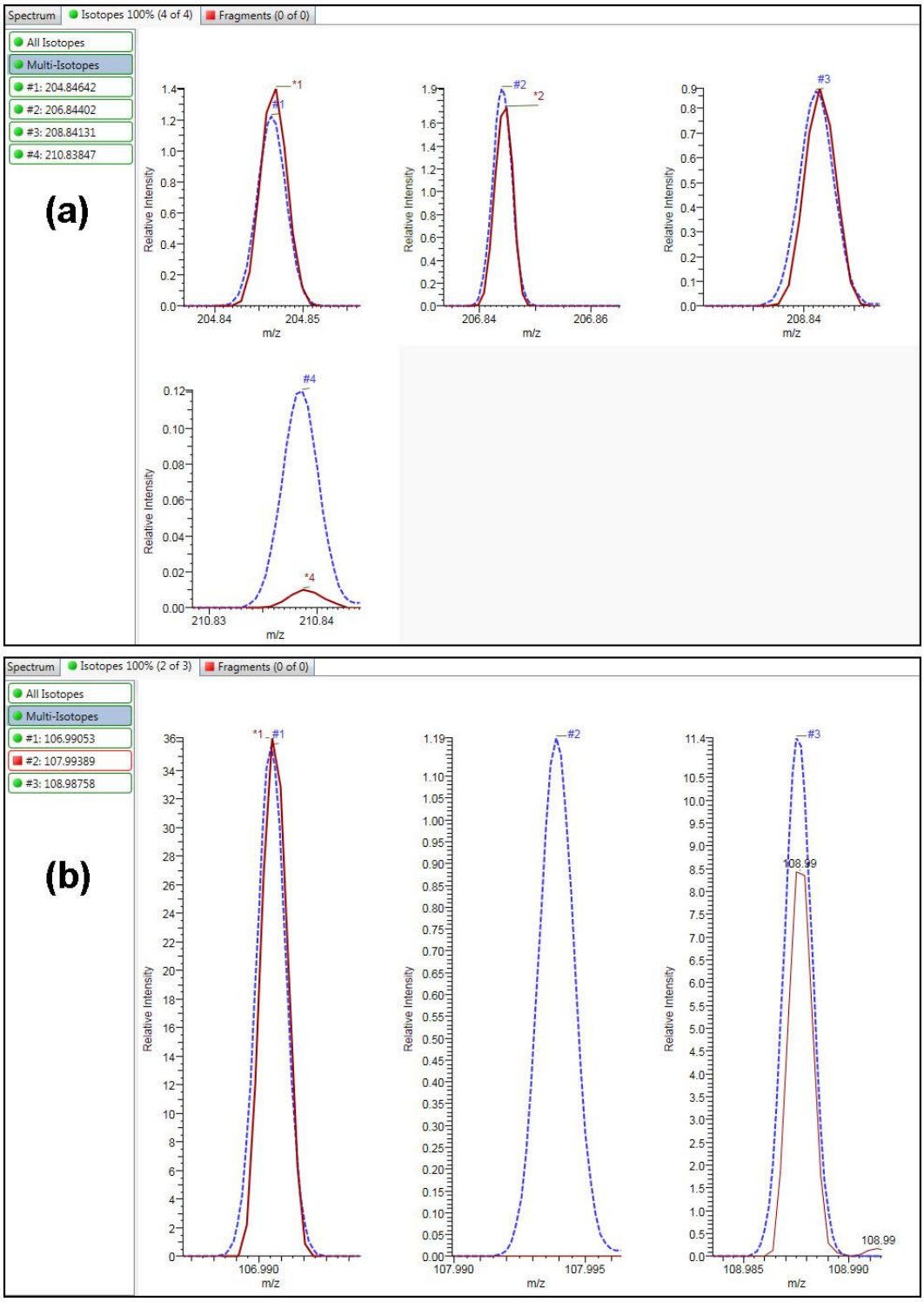

Figure 6 – Automatic comparison using TraceFinder of the isotopic profiles of suspected (a) BDCAA and (b) MCPA detected in a screened drinking-water sample. Both compounds gave a 100 % match.