H₂Open Journal

A validated reverse-phase LC-MS/MS method for the analysis of haloacetic acids in drinking water: supporting the transition from HAA5 to HAA9

Polly L. Grundy [a], Peter R. Jarvis [a], Bruce Jefferson [a], John Fawell[a], John A. Haley[b] and Emma H. Goslan [a,*]

[a] Cranfield University, College Road, Wharley End, Bedfordshire MK43 0AL, UK
[b] UK Water Industry Research Ltd (UKWIR), 1st Floor, 3 More London Riverside, London SE1 2RE, UK
*Corresponding author. E-mail: e.h.goslan@cranfield.ac.uk

PLG, 0000-0003-4899-6954; PRJ, 0000-0003-2740-6740; BJ, 0000-0003-4320-628X; EHG, 0000-0003-0367-226X

ABSTRACT

Haloacetic acids (HAAs) are potentially toxic by-products formed from interactions between organic matter and chlorine during disinfection of drinking water, with brominated HAAs forming when bromide is present. Some countries require monitoring of drinking water for five HAAs, but there is increasing health concern related to the more toxic brominated HAAs and monitoring of nine HAAs (HAA9) is becoming more widespread. However, existing methods of analysis for HAA9 are often sub-optimal, involving complex derivatisation steps and/or long analytical run times. This article presents an improved methodology utilising reverse-phase liquid chromatography mass spectrometry (LC-MS/MS) for which sample preparation involves simple pH adjustment and the analytical run takes 10 min. The efficacy of the method was demonstrated by a full validation across four drinking water matrices with good sensitivity (<0.8 μg/L), precision (<7%), and bias (<10%) observed. A direct comparison using real water samples was performed against the widely used existing gas chromatography method. The new LC-MS/MS method was significantly quicker and easier and demonstrated improved performance in terms of accuracy and precision. This has implications for understanding the risk posed by HAAs in chlorinated water by eliminating the possible historical under-estimates of the levels of the more toxic brominated compounds.

Key words: brominated haloacetic acids, disinfection by-products, HAAs, liquid chromatography mass spectrometry, reverse phase

HIGHLIGHTS

- Presentation of the reverse-phase LC-MS/MS method for nine haloacetic acids in drinking water.
- Full validation and assessment of performance to meet likely regulatory requirements.
- Improvements in recovery and accuracy compared to the existing gas chromatography electron capture detection method.
- Includes implementation protocol to aid widespread adoption.

GRAPHICAL ABSTRACT
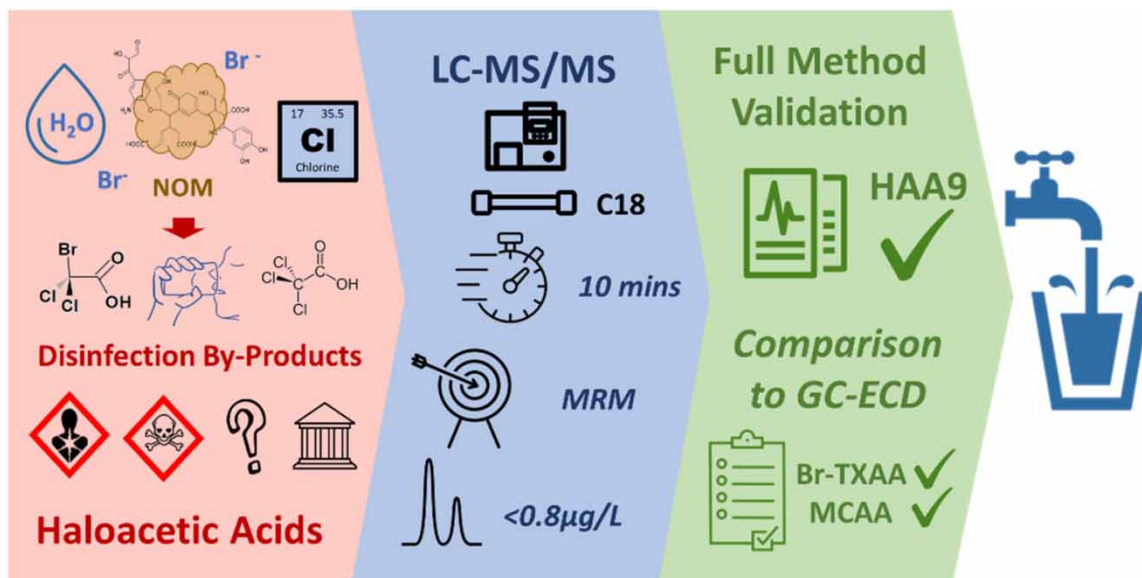
INTRODUCTION

Chlorination was first employed for disinfection and making water safer to drink in the 1890s, and since then its use has saved countless lives due to its ability to inactivate pathogenic organisms. However, chlorine also interacts with the organic matter present in water to form disinfection by-products (DBPs), with brominated as well as chlorinated DBPs formed when bromide is present in the water. DBPs were first identified in the 1970s when chloroform was detected in water supplies (Rook 1974), and since then many hundreds have been identified. Of these, the major classes by mass are trihalomethanes (THMs) and haloacetic acids (HAAs). DBPs are of concern as they have been identified as being potentially geno- and cyto-toxic (Richardson *et al.* 2007; National Toxicology Program (NTP) 2018) and have been associated with an increased incidence of bladder cancer in epidemiological studies (Evans *et al.* 2020). In response to this concern, DBPs are widely regulated, with levels for the four chlorinated and brominated THMs being limited to <100 μg/L in the United Kingdom.

More recently, attention has been turning to haloacetic acids (Table 1) and in particular to the brominated HAAs due to their enhanced toxicity relative to the chlorinated analogues (Richardson *et al.* 2007; Sawade *et al.* 2016). Currently, HAA levels are restricted to <60 μg/L for five HAAs (HAA5) in the United States, European Union (EU), and Scotland, but this excludes some of the brominated HAAs. Given the enhanced toxicity of the brominated HAAs, the monitoring and potential regulation of all nine chlorinated and brominated compounds (nine HAAs (HAA9)) has been discussed in both the EU and the United States (European Commission 2017; Samson & Seidel 2022) and is recommended by the World Health Organisation (WHO) (WHO 2022). Monitoring of all nine haloacetic acids is correspondingly likely to become more relevant as pressure on water resources results in the need to use lower quality source waters or to increase the use of desalinated water, resulting in higher levels of bromide and therefore increased formation of the more toxic brominated DBPs during chlorine disinfection (Chowdhury 2023).

Despite the evidence for the higher toxicity of the brominated HAAs, their current absence from many regulatory frameworks can be explained in part by the assumption that THMs and/or HAA5 can be used as a proxy for HAA9. A more detailed understanding of the formation of DBPs has identified that this assumption may not be valid, with evidence accumulating that although some correlation can be seen between THMs and HAA9 (Samson *et al.* 2017), this cannot be relied upon in all water types and treatment conditions (Malliarou *et al.* 2005; Kolb *et al.* 2017). In addition, their regulatory omission may be related to the analytical challenge they present, with many methods failing to detect all nine compounds adequately, and the non-availability of suitable analytical standards until relatively recently. Therefore, a substantial gap exists in the understanding of the prevalence of the unregulated brominated HAAs, with many studies excluding their analysis due to the difficulties

Table 1 | Haloacetic acids

Name	Abbreviation	CAS	Structure	HAA5
Monochloroacetic acid	MCAA	79-11-8		Y
Monobromoacetic acid	MBAA	79-08-3		Y
Dichloroacetic acid	DCAA	79-43-6		Y
Bromochloroacetic acid	BCAA	5589-96-8		
Dibromoacetic acid	DBAA	631-64-1		Y
Trichloroacetic acid	TCAA	76-03-9		Y
Bromodichloroacetic acid	BDCAA	71133-14-7		
Dibromochloroacetic acid	DBCAA	5278-95-5		
Tribromoacetic acid	TBAA	75-96-7		

of obtaining quality data. It is this absence of a simple and effective validated analytical method and the implications of that shortcoming which this article seeks to address.

The current US Environmental Protection Agency (EPA) methods for HAAs which have been validated for use are EPA 552.3 (Domino *et al.* 2003), which entails derivatisation by methylation, liquid–liquid extraction, and analysis by gas chromatography electron capture detection (GC-ECD) and EPA 557 (Zaffiro *et al.* 2009), which is a direct aqueous injection and analysis by ion chromatography tandem mass spectrometry (IC-MS/MS).

The GC-ECD method (EPA 552.3) is widely used but is time consuming in terms of both extraction (2 h derivatisation plus multiple solvent exchange steps) and analysis (run times up to 1 h). Furthermore, there is dependence on phase separation and derivatisation, both of which can be highly variable, and the low selectivity of the ECD can result in potential false positives. For example, the mono-halogenated HAAs have been seen to extract more efficiently in high ionic strength samples, which can result in incorrectly high quantification for these compounds (Domino *et al.* 2004). Conversely, the derivatisation efficiency for the larger brominated tri-halogenated HAAs (Br-TXAAs) has been identified as being relatively low, with only 45% efficiency for the tri-bromoacetic acid (TBAA) observed (Domino *et al.* 2004). These Br-TXAAs have also been observed to be unstable in the extract solution if peroxide impurities are present, lowering the results obtained (Xie *et al.* 2002). Furthermore, the lack of a specific detector may result in reagent impurities or matrix interference producing false-positive results, with known interferants identified for BCAA (from dimethyl sulphide) and MCAA (TAME impurities) (Domino *et al.* 2003).

The IC-MS/MS method (EPA 557) has been less widely adopted but overcomes many of these issues by removing the need for derivatisation and utilising a highly specific tandem mass spectrometer. However, the method still suffers from some drawbacks. In particular, common background anions, including chlorite, are known to interfere and so have to be carefully separated chromatographically, resulting in a relatively long run time (~1 h) or the risk of high matrix suppression effects. In addition, the HAAs are not stable in the alkaline conditions of the mobile phase, and so a cooled column compartment is required to improve analyte stability (Wu *et al.* 2017; Bruzzoniti *et al.* 2019).

Due to the shortcomings of the existing US EPA methods, a wide range of alternative methodologies have been developed (Ma *et al.* 2024). Proposed analytical strategies range from capillary electrophoresis with ultraviolet detection, to GC-mass spectrometry. A variety of sample preparation techniques have also been put forward, from simple direct injection to complex extractions utilising solid phase cartridges (see Supplementary Material

Table S1). Reverse-phase liquid chromatography tandem mass spectrometry (RP-LC-MS/MS) has the potential to provide a robust and effective analytical method for HAA9 (Duan *et al.* 2011; Alexandrou *et al.* 2019); however, it has hitherto not been as widely adopted. One reason for this is the lack of regulatory imperative to analyse for all nine HAAs, or indeed any HAAs in many countries. This issue is compounded by the current absence of a reverse-phase LC-MS/MS method being approved and validated by the regulatory authorities. Another reason for this omission may be attributed to the fact that the LC-MS/MS instrumentation itself has been perceived to be relatively expensive to purchase and operate, with some less well-resourced laboratories lacking the funds for such equipment. However, these instruments are becoming more affordable and are now relatively commonplace in drinking water testing laboratories in wealthier countries. This is largely due to their high performance and high throughput, and their analytical versatility for contaminants such as pesticides and perfluorinated compounds. Laboratories may also have experienced difficulties during the implementation of the RP-LC-MS/MS method, which requires a degree of technical expertise and the use of modern highly retentive columns due to the analytical issues around detecting such small, polar molecules by this technology. However, once these method development challenges have been overcome and the laboratory demonstrates acceptable performance through a full validation, it should be considered acceptable to regulatory bodies. It is also prudent to investigate the impact of the change in methodology and to provide evidence for an improvement over the status quo. However, no such validation has been published for a reverse-phase LC-MS/MS method for HAA9 nor has a direct comparison to the widely used GC-ECD method previously been undertaken.

This article presents a simple and effective method for the analysis of HAA9 by LC-MS/MS, which is demonstrably fit for purpose for the regulatory analysis of haloacetic acids in drinking water. The implications of changing to such a method from the current widely used GC-ECD method are also assessed by a direct comparison using real drinking water samples, with the hypothesis that the LC-MS/MS method will offer worthwhile improvements. Such a comparison enables the impact of changing methods to be demonstrated, as well as an assessment of the validity of past research. It is intended that the improved analytical method will enable better assessment of the levels of HAAs in drinking water, including the more toxic brominated species, and thus improve our understanding of their prevalence and the risk they pose. This will inform the debate around whether regulation of HAAs, and HAA9 in particular, could or should be adopted more widely.

MATERIALS AND METHODS

Reagents and materials

HAA mixed standard solution (HAA9, each at 1,000 mg/L in methyl tert-butyl ether (MTBE)) was purchased from Restek (Centre County, PA, USA). HAA mixed standard solution (HAA9, each at 2,000 mg/L in MTBE) and Internal Standards 2,3-dibromopropionic acid solution (2,3-DBPA, at 1,000 mg/L in MTBE) and 1,2,3-trichloropropane (TCP; at 200 μg/ml in methanol) were purchased from Merck (Darmstadt, Germany). Methanol (LCMS grade), formic acid (LCMS grade), MTBE (HPLC grade), sodium sulphate (extra pure), sulphuric acid (concentrated, reagent grade), and ammonium chloride (reagent grade) were purchased from Fisher Scientific (Pittsburgh, USA). The mineral water used was Harrogate Spring Water (Harrogate, UK). Ultra-pure water with resistance 18.2 MΩ cm was provided by an Elga Purelab Ultra Genetic system.

Validation was performed using non-chlorinated, treated water from representative UK locations including upland (high organic), lowland, and mixed sources, together with chlorinated tap water from a groundwater source. Method comparison was performed using treated and chlorinated tap water from locations, which included ground and surface water sources.

LC-MS/MS methodology

LC-MS/MS method development

A method for the analysis of HAA9 by reverse-phase LC-MS/MS was developed and validated in house based on the procedure by Duan *et al.* (2011). Optimisation of the method was complicated by the multiple masses and adducts formed by HAAs when analysed by LC-MS/MS, along with the practical requirement to use a mixed analytical standard for HAA9. Each compound has the potential to ionise as the molecular ion [M-H]⁻ but also as the [M-COOH]⁻ decarboxylated ion. Combined with the potential isotopes of ^{35}Cl, ^{37}Cl, ^{79}Br, and ^{81}Br results in a large number of possible precursor (parent) ions and even more product (daughter) ions, with many of these masses overlapping between the target compounds. Consideration of the 9 HAAs gives 150 possible mass transitions (precursor to product ion), and of these, 31 occur for more than one compound (see

Supplementary Material Table S6 for all likely mass transitions). Therefore, a cautious approach to the method development is necessary and was adopted (detailed in the Supplementary Material Figure S1 and Table S3). This enabled the HAAs to be separated chromatographically before the selection of unique qualifier and quantifier transitions for each compound, which were then optimised for maximum sensitivity and selectivity. The optimised method, as detailed here and in the Supplementary Material, was submitted to full validation as utilised by UK drinking water laboratories when demonstrating method performance for regulatory purposes. A comparison to the existing GC-ECD method was also performed.

LC-MS/MS standard preparation

Intermediate standards of HAA9 and 2,3-DBPA were prepared at 1 mg/L in ultra-pure water and stored in the dark in the fridge for a maximum of 2 months (as detailed in EPA 557). Calibration solutions were prepared fresh with each batch at concentrations from 0.5 to 100 μg/L in the matrix-matched solution. The matrix-matched solution was non-chlorinated potable water (bottled mineral water) containing ammonium chloride preservative and adjusted to a pH of 2 ± 0.5 in the same manner as a sample.

LC-MS/MS sample preparation

Samples were preserved immediately at collection with 100 mg/L ammonium chloride and stored in the fridge in the dark for up to two weeks. Samples were then adjusted to pH 2 ± 0.5 with 10% sulphuric acid. Internal standard 2,3-DBPA was added to samples and standards at 10 μg/L and the relative response used for quantification.

LC-MS/MS analysis

The instrument used was a SCIEX (Framingham, MA, USA) ExionLC ultra high-pressure liquid chromatography (UHPLC) system with temperature-controlled autosampler and column compartment and Qtrap 6500+ mass spectrometer system with electrospray ionisation source. The chromatography column used was a Waters (Milford, MA, USA) Acquity HSS T3 Premier 100×2.1 mm 1.8 μm with the associated Vanguard column. The mobile phase comprised mobile phase A (0.2 mM formic acid in water) and mobile phase B (0.2 mM formic acid in methanol). Initial conditions were 1% B for 1 min, followed by a gradient to 70% B at 5 min then holding for 2 min before re-equilibration, with a total run time of 10 min. The flow was 0.2 ml/min, injection volume was 20 μL, column temperature was 50 °C, and autosampler temperature was 7 °C.

The mass spectrometer was operated in the negative ionisation multiple reaction monitoring (MRM) mode. Nitrogen curtain gas (CUR) was set to 25 psi, ion source temperature (TEM) 350 °C, compressed air ion source gas 1 (GS1) 50 psi, ion source gas 2 (GS2) 60 psi, nitrogen collisionally activated dissociation gas (CAD) was 'high', and ion spray voltage (IS) −4,500 V. The instrument was controlled with SCIEX Analyst version 1.6.3 software, and the quantification was performed with SCIEX MultiQuant version 3.0.2. The acquisition parameters and observed retention times for individual compounds are summarised in Table 2, and further details are provided in the Supplementary Material Table S4.

Table 2 | LC-MS/MS acquisition parameters

Compound	Retention time (min)	Q1 m/z (Da)	Q3 m/z (Da)	Q1 m/z (Da)	Q3 m/z (Da)
MCAA	2.80	93.0	35.0	95.0	37.0
MBAA	3.45	136.9	78.9	138.9	80.9
DCAA	4.12	126.9	35.0	126.9	83.0
BCAA	4.48	126.9	78.9	170.9	126.9
DBAA	4.85	216.8	172.8	216.8	78.9
TCAA	6.00	116.9	35.0	118.9	37.0
BDCAA	6.18	160.9	78.9	162.9	80.9
DBCAA	6.34	206.8	78.9	208.8	80.9
TBAA	6.50	252.8	80.9	250.8	78.9
2,3-DBPA[a]	5.76	80.9	80.9	78.9	78.9

[a]IS, internal standard.

GC-ECD methodology

The GC-ECD method used was a modified version of EPA 552.3 (Domino *et al.* 2003; Tung *et al.* 2006).

GC-ECD standard preparation

An intermediate standard solution of HAA9 in ultra-pure water was prepared at 200 µg/L and used to prepare a set of calibration standards, all 30 ml in ultra-pure water, at concentrations from 1 to 100 µg/L. These were prepared fresh for each batch and extracted as per samples.

GC-ECD sample preparation

Samples were preserved immediately at collection with 100 mg/L ammonium chloride and stored in the fridge for up to 2 weeks. A sample of 30 ml was acidified using 1.5 ml sulphuric acid before addition of 3 ml MTBE containing 1,000 µg/L TCP internal standard and approximately 12 g sodium sulphate. Samples were shaken vigorously for 3 min before allowing phase separation. One millilitre of the upper MTBE layer was combined with 1 ml 10% sulphuric acid in methanol in a test tube and placed in a water bath at 50 °C for 2 h. Once cooled, 1 ml MTBE and 3 ml 10% sodium sulphate (aqueous) were added and mixed before removing the lower aqueous layer and discarding. A further 1 ml of 10% sodium sulphate was added and mixed before taking the upper MTBE layer for analysis.

GC-ECD analysis

The instrument used was a Hewlett-Packard 6890 GC-ECD (Agilent, Santa-Clara, CA, USA). The chromatography column was a Restek (Centre County, PA, USA) Rtx-1 30 m, 250 µm diameter, 1 µm thickness. The injection volume was 1 µL, and the injector was run in the split mode at 200 °C 12.7 psi with a split ratio 10:1. The initial oven temperature was 40 °C with a hold time of 5 min followed by ramp at 4 °C/min to 100 °C, then a hold time for 1 min before ramping at 8 °C/min to 200 °C, and then a hold time of 3 min before returning to 40 °C for post-run time of 5 min. The carrier gas was helium with an initial pressure of 12.7 psi and a flow of 1.1 ml/min. The ECD was operated at a temperature of 270 °C with nitrogen at 30 ml/min. The instrument was controlled, and quantitation was performed with OpenLab CDS Chemstation version C.01.07 SR4 software.

The observed retention times for the individual compounds are provided in Supplementary Material Table S5. The relative response to the internal standard was used for quantification.

Validation protocol

The LC-MS/MS method was validated in line with the recommendations of the UK Drinking Water Inspectorate (DWI) and ISO:17025 standards, and in compliance with NS30 and UK Drinking Water Testing Specification (DWTS) single laboratory validation with at least 10 degrees of freedom.

As such, 11 batches of duplicate samples were extracted and analysed separately, with each batch comprising neat, low spiked (1 µg/L) and high spiked (10 µg/L) samples from four water types as well as duplicate standards at 20 and 80% of the method range. Representative matrices were selected from a range of water types including lowland water, upland water, impounding reservoir water, and chlorinated groundwater.

Validation performance targets were based on a possible prescribed concentration value (PCV) of 80 µg/L for the sum of HAA9, in line with that originally proposed by the EU (European Commission 2017). Other validation performance targets are in line with UK DWI recommendations for THMs, in that the limit of detection (LOD) should be less than 10% of PCV, and trueness and precision should be less than 25%. Method uncertainty was calculated as recommended by the UK Standing Committee of Analysts (SCA), where

$$u_c = \sqrt{u_R^2 + u_b^2}$$

where u_c is the combined standard uncertainty, u_R is the precision as relative standard deviation, u_b is the percent bias, and the expanded uncertainty is u_c multiplied by a coverage factor of $k = 2$ to give a confidence level of approximately 95% (SCA 2021).

Protocol for direct comparison of method performance

The new LC-MS/MS method and the widely used existing GC-ECD method were directly compared using real world treated, chlorinated tap water selected from six different locations including ground and surface water sources. These samples were used directly and to prepare fortified samples by addition of 2 and 20 µg/L of

each HAA, thus ensuring a range of levels and matrices were assessed. A total of 36 samples were prepared this way and then analysed by both methods simultaneously to enable direct method comparison.

Protocol for quality assurance and quality control

Once validated, the ongoing quality of the results obtained was assured using appropriate quality control and assurance procedures. As such, a calibration set covering the reporting range of the method was prepared with each batch and analysed prior to any samples, with a mid-range check standard at least every 20 injections and at the end of the batch. Correlation was required to be $r^2 > 0.995$ and check standards within 15% of nominal. Samples outside of the calibrated range were not extrapolated but repeated with dilution. Blanks were prepared as if samples using ultra-pure water and determined to be <1/3 reporting limit. Spiked (fortified) samples containing 10 μg/L HAA9 were prepared for every matrix type and at least every 10 samples, with acceptable recoveries within 80–120%. Duplicate samples were prepared at least every 20 samples with results within 20% or 10 μg/L. Internal standard response was monitored and acceptance limited to within 60–140% of that obtained in calibration standards. The ratio between qualifier and quantifier transition for each analyte was monitored using the software, and failures were identified if the ratio was >20% different from that achieved in the calibration. Peak shape and resolution between 2,3-DBPA and BDCAA were monitored and used to identify deterioration in column performance and the need for corrective action (column clean or change).

RESULTS AND DISCUSSION

LC-MS/MS method performance

The LC-MS/MS method was developed using the described protocol (see Supplementary Material) to ensure a method with good chromatography and spectrometry was achieved. This includes full resolution of BDCAA and 2,3-DBPA and two MRM transitions for quantitation and qualification of each analyte. Subsequent validation in drinking water matrices revealed that the performance exceeded standard requirements and thus can be deemed an appropriate method for HAA9 analysis in drinking water (Table 3).

Linearity and range

Linearity was demonstrated across the range of 0.5–100 μg/L using matrix-matched calibration standards and duplicate analyses at 20 and 80% of the range. The correlation coefficient r^2 for the calibration curves was greater than the target of 0.995 and exceeded 0.999 in all cases. The precision at 20 and 80%, measured as 2× relative standard deviation, met the target of <25% in all cases, and the trueness, measured as bias, met the requirement of $< \pm 25\%$ and was <5% in all cases.

Sensitivity and limit of detection

The LOD of the method was calculated as 3× standard deviation of non-chlorinated samples spiked at 1 μg/L. In all cases, the LOD was less than the target of 0.8 μg/L (<10% of the PCV).

Table 3 | Summary of validation performance for LC-MS/MS method

Compound	LOD, μg/L	Recovery ± RSD (%)		Linearity, r^2	Expanded uncertainty[a], %
		1 μg/L	10 μg/L		
MCAA	0.37	90.6 ± 6.8	97.2 ± 3.3	0.99958	14.12
MBAA	0.16	100.7 ± 2.7	100.2 ± 2.2	0.99963	8.73
DCAA	0.18	96.6 ± 3.1	102.1 ± 2.1	0.99961	9.2
BCAA	0.12	99.6 ± 2.0	100.3 ± 2.1	0.99972	8.34
DBAA	0.16	102.7 ± 2.6	101.8 ± 1.8	0.99965	8.13
TCAA	0.28	98.8 ± 4.6	104.7 ± 2.3	0.99954	13.06
BDCAA	0.17	102.4 ± 2.8	102.6 ± 2.8	0.99936	12.46
DBCAA	0.14	101.6 ± 2.4	101.9 ± 1.7	0.99956	7.64
TBAA	0.14	102.1 ± 2.2	102.8 ± 1.8	0.99962	9.08

[a]The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor of $k = 2$ to give a confidence level of approximately 95%.

Precision and selectivity

The precision was calculated as 2× relative standard deviation of spiked samples at around the PCV and in all cases found to be significantly less than the target of 25%. The selectivity was demonstrated using two transitions for each compound, which gives a high degree of certainty due to the presence of separate precursor and product ions for each. In addition chromatographic resolution for overlapping transitions was required and in particular for 2,3-DBPA and BDCAA (see examples in Supplementary Material Figure S2). It was observed that no false positives were identified in known negative samples and that known positive samples demonstrated passing ion ratios in all cases.

Accuracy and bias

Analysis of spiked samples across the four matrices demonstrated recoveries within the range 90–110%, which is well within the target of 75–125%.

Method uncertainty

Expanded method uncertainty was calculated based on a 95% confidence limit and found to be <15% for all analytes. This compares with the published US EPA methods, where the expanded uncertainty (calculated from the published validation data) was between 2 and 26% for EPA 552.3 and 4 and 20% for EPA 557 (data from Domino et al. 2003; Zaffiro et al. 2009).

Comparison to GC-ECD

Thirty-six samples were analysed in parallel by the validated LC-MS/MS method and the existing EPA 552.3 GC-ECD method, covering six water sources and including extracts of fortified samples from each water source. Observation of the time required for both extraction and analysis by each method, as well as the requirement for solvents and other reagent chemicals, demonstrated that the LC-MS/MS method is substantially quicker and involved lesser amounts of potentially hazardous and environmentally damaging chemicals. To enable better comparison of the practical considerations for each analytical option for HAA9, this assessment was quantified for a hypothetical testing laboratory performing 20 samples per week and extended to encompass the other alternative validated method, EPA 557 (Supplementary Material Table S2). This review confirmed the potential of the LC-MS/MS method to reduce laboratory sample preparation time and reagent use compared to GC-ECD, as well as making optimal use of mass spectrometry equipment when compared to EPA 557. However, it highlighted that capital and operational costs would be significantly higher for both LC-MS/MS and EPA 557 than for the GC-ECD method.

Assessment of the analytical results obtained from the real-world sample comparison shows that for unfortified waters where HAA levels are low (less than 20 μg/L), the two techniques demonstrate agreement across the different water types to within 3 μg/L in all bar one case (Table 4).

The major difference identified in the results was the reporting of a significantly higher result for MCAA in Sample 5 by GC-ECD (21.7 μg/L as opposed to <0.8 μg/L by LC-MS/MS). Further divergence in the methods was observed in the measured performance parameters, whereby overall recovery across the nine HAAs was 102.7% for the LC-MS/MS method and 85.4% for the GC-ECD method, with the average relative standard deviation at 6.1% and 13.1%, respectively (Figure 1). In addition, the sensitivity of the methods to MCAA was not equal, with reporting limits <5 μg/L by GC-ECD and <0.8 μg/L by LC-MS/MS, respectively.

The difference in recovery was most pronounced for BDCAA, DBCAA, and TBAA, where observed recoveries by the LC-MS/MS method were 105.9, 107.0, and 107.2%, respectively, compared to 68.2, 65.3, and 67.8% by GC-ECD. Importantly, in the case of the GC-ECD method, for these compounds, the recovery values are less than the target of ±25% trueness (recovery 75–125%) set for validation, and therefore, from this dataset, they would be excluded from regulatory reporting. The previous research has identified that the derivatisation efficiency of brominated tri-halogenated acetic acids is lower than for the smaller HAAs (Domino et al. 2004), and these results suggest that the derivatisation efficiency is further adversely affected by the matrix, resulting in lower values when compared with the calibration standards (which are derivatised in ultra-pure water and therefore have a minimal matrix affect).

In addition, differences were observed with respect to MCAA, which reported a lower mean recovery of 76% and a large variance between samples when using the GC-ECD method compared to the LC-MS/MS method. Significantly, Sample 5 reported a concentration of 21.7 μg/L with the GC-ECD method, which was identified

Table 4 | Method comparison in samples

Compound	Source type/method	1 Surface	2 Ground	3 Surface	4 Mixed	5 Surface	6 Ground
		Sample result, μg/L					
MCAA	LC-MS/MS	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
	GC-ECD	<5	<5	<5	<5	21.7	<5
MBAA	LC-MS/MS	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
	GC-ECD	<1	<1	<1	<1	<1	<1
DCAA	LC-MS/MS	<0.8	<0.8	2.5	1.2	0.9	<0.8
	GC-ECD	1.3	<1	5.4	1.8	2.8	<1
BCAA	LC-MS/MS	<0.8	<0.8	2.3	1.6	2.4	<0.8
	GC-ECD	1.2	<1	3.3	1.7	3.5	<1
DBAA	LC-MS/MS	<0.8	2.3	1.5	2.4	4.4	<0.8
	GC-ECD	1.3	2.3	1.5	1.9	4.7	<1
TCAA	LC-MS/MS	<0.8	<0.8	2.6	<0.8	<0.8	<0.8
	GC-ECD	<1	<1	2.5	<1	<1	<1
BDCAA	LC-MS/MS	1.3	<0.8	3.4	0.8	1	<0.8
	GC-ECD	<1	<1	2	<1	<1	<1
DBCAA	LC-MS/MS	2.1	<0.8	2.1	1.8	2.4	<0.8
	GC-ECD	1.2	<1	1.2	1.1	1.4	<1
TBAA	LC-MS/MS	<0.8	<0.8	<0.8	0.8	1.2	<0.8
	GC-ECD	<1	<1	<1	<1	<1	<1
Total HAA9	LC-MS/MS	*< 8*	*< 8*	*15.5*	*9.2*	*13.2*	*< 8*
	GC-ECD	*< 13*	*< 13*	*16.1*	*< 13*	*36.4*	*< 13*
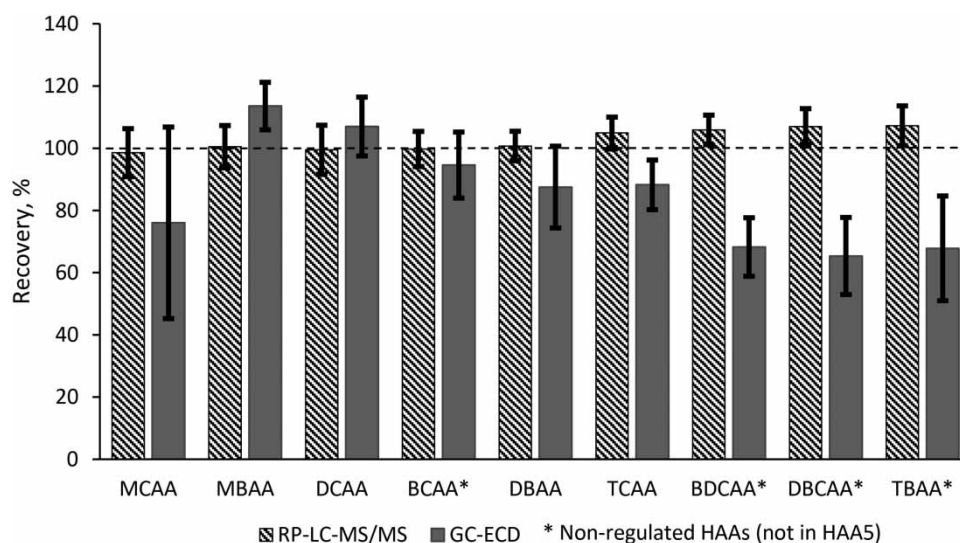
Figure 1 | Comparison in performance between LC-MS/MS and GC-ECD methods, with error bars demonstrating relative standard deviation.

as a false positive caused by a co-eluting impurity. Issues with measuring MCAA have been previously reported whereby it has not been possible to report MCAA due to analytical issues (Malliarou *et al.* 2005) or where unexpectedly large values have been seen for MCAA in real water samples (Simpson & Hayes 1998; Avşar & Kılıç 2023). Given that the expectation is for a low formation potential of MCAA in most circumstances (thought to be due to the compounding effect of promotion of electrophilic substitution and low preference for hydrolysis from singly halogenated intermediates (Liang & Singer 2003; Weng *et al.* 2022)), it raises the likelihood that the GC-ECD method is sometimes leading to overestimation of MCAA levels, which requires further investigation.

Further method appraisal

In addition to the comparisons to the approved US EPA methods, our proposed method can be assessed against some of the alternative available methods. Efforts to overcome the obstacles presented by the EPA methods have resulted in a wide range of methods being published, many of which include the benefits of speed and accuracy such as offered by reverse-phase LC-MS/MS, but also have their own inherent downsides (Table S1).

GC-based methods have been developed, which improve selectivity by utilising mass spectrometry (Franco et al. 2019) or increase speed by using microextraction techniques (Wu Gabryelski & Froese 2002). However, although these do offer improvements, they do not overcome the inherent problem that haloacetic acids require derivatisation to be suitable for the GC analysis. Not only does this introduce a time and cost factor by requiring a sample preparation step but it also increases the risk of introducing a negative bias towards the brominated tri-halogenated HAAs.

LC-based methods remove the need for derivatisation, but in many cases, the sensitivity required means that sample preparation is needed to increase concentrations and remove interfering matrices. This increases the time for sample preparation and potential for losses, but can allow for more complex matrices to be analysed (Duan et al. 2013; Alsharaa et al. 2016). In contrast, the direct injection reverse-phase chromatography presented here would be unlikely to adapt to wastewater analysis due to the high susceptibility of LC-MS/MS to matrix suppression.

Direct analysis methods, such as direct injection liquid or ion chromatography, can reduce sample extraction errors and delays by minimising the sample preparation, but generally require expensive detection systems. Despite using high-end detectors, both LC and IC-MS/MS are prone to matrix interference by co-eluting organic matter and anions, respectively. This can be overcome by selective, fully optimised chromatography. For ion chromatography, this often entails using an alkali mobile phase and long-run times, which decreases the stability of the HAAs during analysis (Bruzzoniti et al. 2019). For the reverse phase, some methods have utilised ion pair agents to improve retention (Takino Daishima & Yamaguchi 2000). This enables better separation, but there are downsides, which include lower sensitivity due to suppression in the mass spectrometer and the potential for a long-term impact on mass spectrometer performance due to buildup of ion pair agents. Other alternatives include two-dimensional chromatography, which improves separation from background contamination but adds an extra layer of cost and complexity to the analysis (Wagner et al. 2017).

Therefore, reverse-phase chromatography using specialised polar columns, such as this method presents, remains a useful option in low organic matrices such as drinking water. Comparisons to other published direct injection reverse-phase LC-MS/MS methods confirm that it has the potential to efficiently provide accurate results, with Duan et al. (2011) demonstrating comparable method performance albeit over a much smaller sample size. However, it is observed that if chromatography is not properly optimised, this has implications for data quality (for example, an expanded uncertainty of up to 116% was obtained for MCAA under less optimal LC-MS/MS conditions (Planas et al. 2019)). Other useful applications of reverse-phase methods include utilising high-resolution mass spectrometry (HRMS), which has a distinct advantage over MS/MS in the ability to identify non-target DBPs in the same analytical run. However, they are equally if not more expensive and generally have lower sensitivity than the tandem mass spectrometers (Planas et al. 2019).

Further considerations when comparing methods include the ability to combine with other analyses if required. The method presented here could be expanded to include other, small, negatively charged target analytes potentially encompassing emerging contaminants such as the smaller perfluorinated acids, pesticide metabolites, or iodinated HAAs. However, because the analytical run is in negative ionisation mode and includes a relatively slow, aqueous gradient, it will not be readily combined with other drinking water LC-MS/MS testing suites (most pesticides and other contaminants will run faster and in positive mode). Also, unlike most HRMS, the data are purely targeted and cannot be re-visited retrospectively to investigate as yet unknown contaminants.

Implications of findings

The results of the current study reveal that adopting the reverse-phase LC-MS/MS method outlined in this article improves measurement accuracy and reduces the risk of false positives for all the HAAs, not just MCAA. This is a result of the additional selectivity of the MS/MS detector when compared to the ECD due to the multiple mass transitions used and a further degree of certainty due to the good spike recoveries in real matrices. In addition, the reduction in time associated with sample preparation and analysis affords the potential for the inclusion of enhanced quality checks, such as spiked or duplicate samples within each analytical batch, imparting further confidence in the data obtained.

Critically, the LS-MS/MS method provides a route to faster, more reliable, and confident measurement of the larger, brominated tri-halogenated haloacetic acids. Accordingly, the use of this method affords the opportunity to switch to the measurement of all nine haloacetic acids, as opposed to the current five, as a means of better understanding the risk associated with DBPs. The importance of this relates to the higher relative toxicity of the brominated species, which are reported to have significantly increased impacts even at low mass concentrations (Plewa *et al.* 2010). For instance, BCAA and BDCAA have been indicated to have toxicity levels significantly above those of the regulated HAAs and THMs (Wagner & Plewa 2017; Lau *et al.* 2023). The current work revealed low recoveries in drinking water matrices for brominated tri-halogenated compounds when using GC-ECD derivatisation-based methods, so it is reasonable to assume that historical underestimation could have occurred, and reassessment of the importance of brominated HAAs is required. For example, the impact of changing methods from GC-ECD to LC-MS/MS on the assessment of the levels of the compounds within HAA9 but not covered by HAA5 (herein called non-regulated HAAs) is significant. The LC-MS/MS method gives consistently higher results for the non-regulated HAAs in the six representative matrices used (Figure 2(a)). Furthermore, if a risk assessment approach is taken using the guideline values to assess the hazard index of the water sources (Goslan *et al.* 2020; WHO 2022), this under-reporting will result in consistent under-estimation of the hazard presented by the water when utilising GC-ECD (Figure 2(b)). This would be of particular concern in source waters with high bromide levels, which represent the greatest potential to form significant concentrations of the more toxic brominated haloacetic acids.

The adoption of this LC-MS/MS method will therefore have particular significance for lowland waters, which have traditionally been of lesser concern for DBP formation due to the relatively low THM levels observed (Whitaker *et al.* 2003). However, many such lowland sources have the possibility of containing significant levels of bromide, and therefore, the potential of such waters to form enhanced levels of the more toxic brominated HAAs exists. Moreover, HAA precursor organic matter molecules are often different from those associated with THM formation and can be more hydrophilic and thus recalcitrant to removal by coagulation and subsequent water treatment processes (Li *et al.* 2014). This further increases the likelihood that the DBP-associated risks of lowland waters may have been historically under-estimated when brominated HAAs were not considered (Lau *et al.* 2023). Similarly, the choice of analytical method will be significant if more complex water matrices are required to be used for water supply as climate change and environmental considerations impact water resource availability. For example, during indirect water re-use, treated chlorinated effluent may be discharged to the environment to replenish drinking water sources, and this can contain significant levels of HAAs (Munné *et al.* 2023). The impact of the higher levels of the matrix in such samples would increase the possibility of underestimation of the risk due to larger, brominated HAAs if the derivatisation-based GC-ECD method was used. Utilising LC-MS/MS would allow the ready use of spiked samples, affording additional confidence in the results and a better appreciation of the risks associated with such emerging practices.
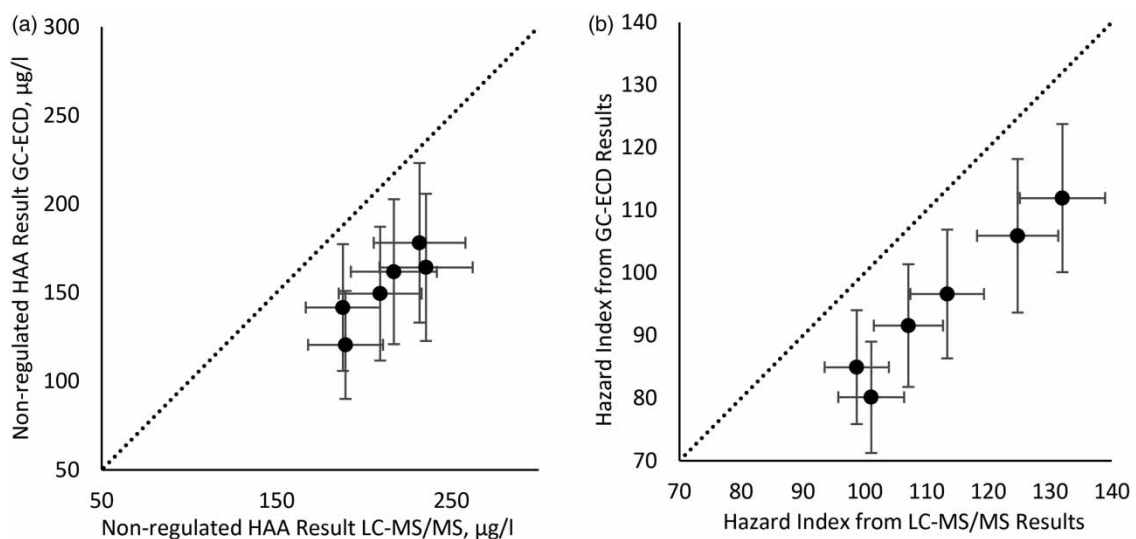

Figure 2 | Parity plots demonstrating influence of changing method on (a) non-regulated HAA results and (b) hazard index from the six water sources, with error bars representing relative standard deviation.

As the water sector moves beyond THMs and embraces routine consideration of HAAs, it is posited that the inclusion of all nine HAAs is prudent to ensure as complete a picture as possible is considered when establishing management strategies to minimise the risk associated with DBPs. This is most apparent when we extend beyond mass concentration to also incorporate relative toxicity through the lens of hazard indices (Goslan *et al.* 2020). This reveals the relatively high importance of low concentrations of specific compounds, most notably the brominated haloacetic acids. Consequently, inclusion of all nine HAA species has the potential to modify our understanding of the most appropriate ways to minimise the risk associated with DBPs. Thus, it is posited that the routine measurement of all nine HAAs is the most appropriate course of action going forward. While equivalent arguments could be made for other emerging DBPs (e.g., nitrogenous DBPs, iodinated DBPs), it is contended that HAA measurement is starting to become routine already and that the required LC-MS/MS is becoming more commonplace. This reflects the utility of such an instrument not only for HAA analysis but also when measuring other micropollutants such as specific pesticides and per and polyfluorinated compounds. It is further proposed that additional utilisation can be obtained by expanding the method presented here to include polar, acidic compounds such as dalapon (2,2-dichloropropionic acid) or herbicide metabolites (Postigo *et al.* 2020).

CONCLUSIONS

In support of the advisability of controlling all nine haloacetic acids, this article proposed a reverse-phase LC-MS/MS method for their measurement and compared its efficacy to the currently used US EPA GC-ECD method. The LC-MS/MS method was fully validated across four representative drinking water matrices, and good sensitivity (<0.8 μg/L), precision ($<7\%$), and bias ($<10\%$) were observed for all nine HAAs. The comparison to GC-ECD demonstrated improved performance by the new method, particularly with regards to the brominated tri-halogenated HAAs, which appear to be under-reported by GC-ECD with low recoveries. Consequently, it is posited that adoption of the presented method is critical in establishing, with appropriate accuracy, the current prevalence, and then future approaches to minimising the risk posed by haloacetic acids.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTION

Polly Grundy: visualisation, investigation, methodology, formal analysis, data curation, validation, writing – original draft, and writing – review and editing. Emma Goslan: conceptualisation, supervision, funding acquisition, and writing – review and editing. Peter Jarvis: conceptualisation, supervision, funding acquisition, and writing – review and editing. Bruce Jefferson: conceptualisation, supervision, funding acquisition, and writing – review and editing. John Fawell: writing – review and editing. John Haley: supervision and writing – review and editing.

CONFLICT OF INTEREST

There are no conflicts to declare.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Alexandrou, L. D., Bowen, C. & Jones, O. A. 2019 Fast analysis of multiple haloacetic acids and nitrosamines in recycled and environmental waters using liquid chromatography-mass spectrometry with positive–negative switching and multiple reaction monitoring. *Analytical Methods* **11** (30), 3793–3799. https://doi.org/10.1039/C9AY01276A.

Alsharaa, A., Sajid, M., Basheer, C., Alhooshani, K. & Lee, H. K. 2016 Determination of haloacetic acids in water using layered double hydroxides as a sorbent in dispersive solid-phase extraction followed by liquid chromatography with tandem mass spectrometry. *Journal of Separation Science* **39** (18), 3610–3615. https://doi.org/10.1002/jssc.201600305.

Avşar, E. & Kılıç, A. 2023 Monitoring seasonal variations of haloacetic acids (HAAS) in low-TOC and low-chlorine networks and assessing risk to public health: Muş, Türkiye case. *Water SA* **49**, 301–310. https://doi.org/10.17159/wsa/2023.v49.i3. 4004.

Bruzzoniti, M. C., Rivoira, L., Meucci, L., Fungi, M., Bocina, M., Binetti, R. & Castiglioni, M. 2019 Towards the revision of the drinking water directive 98/83/EC. Development of a direct injection ion chromatographic-tandem mass spectrometric method for the monitoring of fifteen common and emerging disinfection by-products along the drinking water supply chain. *Journal of Chromatography A* **1605**, 360350. https://doi.org/10.1016/j.chroma.2019.07.004.

Chowdhury, S. 2023 Evaluation and strategy for improving the quality of desalinated water. *Environmental Science and Pollution Research* **30** (24), 65947–65962. https://doi.org/10.1007/s11356-023-27180-1.

Domino, M. M., Pepich, B. V., Munch, D. J., Fair, P. S. & Xie, Y. 2003 EPA METHOD 552.3: Determination of haloacetic acids and dalapon in drinking water by liquid–liquid micro-extraction, derivatization and gas chromatography with electron capture detection, Revision 1.0. Available from: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=901V0400.txt.

Domino, M. M., Pepich, B. V., Munch, D. J. & Fair, P. S. 2004 Optimizing the determination of haloacetic acids in drinking waters. *Journal of Chromatography A* **1035** (1), 9–16. https://doi.org/10.1016/j.chroma.2004.02.034.

Duan, J., Li, W., Si, J. & Mulcahy, D. 2011 Rapid determination of nine haloacetic acids in water using ultra-performance liquid chromatography-tandem mass spectrometry in multiple reactions monitoring mode. *Analytical Methods* **3**, 1667–1673. https://doi.org/10.1039/C1AY05122A.

Duan, J., Li, W., Sun, P., Lai, Q., Mulcahy, D. & Guo, S. 2013 Rapid determination of nine haloacetic acids in wastewater effluents using ultra-performance liquid chromatography-tandem mass spectrometry. *Analytical Letters* **46** (4), 569–588. https://doi.org/10.1080/00032719.2012.729238.

European Commission. 2017 Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the quality of water intended for human consumption (recast). Available from: https://eur-lex.europa.eu/legal-content/ EN/TXT/?qid=1531209398530&uri=CELEX:52017PC0753 (accessed 8 July 2022).

Evans, S., Campbell, C. & Naidenko, O. V. 2020 Analysis of cumulative cancer risk associated with disinfection byproducts in United States drinking water. *International Journal of Environmental Research and Public Health* **17** (6), 2149. https://doi. org/10.3390/ijerph17062149.

Franco, E. S., Pádua, V. L., Rodriguez, M. D. V. R., Silva, D. F., Libânio, M., Pereira, M. C., Silva, P. H. G., Santanta Júnior, I. C., Rocha, B. A., Camargo, J. A., Mourão, A. O. & Rodrigues, J. L. 2019 A simple liquid-liquid extraction-gas chromatography-mass spectrometry method for the determination of haloacetic acids in environmental samples: Application in water with *Microcystis aeruginosa* cells. *Microchemical Journal* **150**, 104088. https://doi.org/10.1016/j.microc.2019.104088.

Goslan, E., Jarvis, P., Fawell, J. K., Losty, E. & Jefferson, B. 2020 *Identification of Treatment Conditions Which Minimise DBP Formation 20/TX/05/3*. 20/TX/05/3. United Kingdom Water Industry Research. Available from: https://ukwir.org/water-research-reports-publications-viewer/d8765ad1-0b86-47db-b5ef-bbcf2917c656.

Kolb, C., Francis, R. A. & VanBriesen, J. M. 2017 Disinfection byproduct regulatory compliance surrogates and bromide-associated risk. *Journal of Environmental Sciences* **58**, 191–207. https://doi.org/10.1016/j.jes.2017.05.043.

Lau, S. S., Tang, T., Odeh, L., Bokenkamp, K., Wagner, E. D., Plewa, M. J. & Mitch, W. A. 2023 Is genotoxicity associated with disinfection byproducts in potable reuse and conventional drinking water additive? *Environmental Science & Technology Letters* **10** (11), 1075–1081. https://doi.org/10.1021/acs.estlett.3c00655.

Li, A., Zhao, X., Mao, R., Liu, H. & Qu, J. 2014 Characterization of dissolved organic matter from surface waters with low to high dissolved organic carbon and the related disinfection byproduct formation potential. *Journal of Hazardous Materials* **271**, 228–235. https://doi.org/10.1016/j.jhazmat.2014.02.009.

Liang, L. & Singer, P. C. 2003 Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. *Environmental Science & Technology* **37** (13), 2920–2928. https://doi.org/10.1021/es026230q.

Ma, W., Li, W., Chen, B., Yang, Y. & Yang, J. 2024 A critical review of chromatographic methods for analyzing haloacetic acids in water: Advances, uncertainties, and prospects. *TrAC Trends in Analytical Chemistry* **173**, 117617. https://doi.org/10. 1016/j.trac.2024.117617.

Malliarou, E., Collins, C., Graham, N. & Nieuwenhuijsen, M. J. 2005 Haloacetic acids in drinking water in the United Kingdom. *Water Research* **39** (12), 2722–2730. https://doi.org/10.1016/j.watres.2005.04.052.

Munné, A., Solà, C., Ejarque, E., Sanchís, J., Serra, P., Corbella, I., Aceves, M., Galofré, B., Boleda, M. R., Paraira, M. & Molist, J. 2023 Indirect potable water reuse to face drought events in Barcelona city. Setting a monitoring procedure to protect aquatic ecosystems and to ensure a safe drinking water supply. *Science of the Total Environment* **866**, 161339. https://doi. org/10.1016/j.scitotenv.2022.161339.

National Toxicology Program (NTP). 2018 *Report on Carcinogens Monograph on Haloacetic Acids Found as Water Disinfection By-Products*. National Institute of Environmental Health Science, Durham, NC. https://doi.org/10.22427/ROC-MGRAPH-12.

Planas, C., Palacios, Ó., Ventura, F., Boleda, M. R., Martín, J. & Caixach, J. 2019 Simultaneous analysis of 11 haloacetic acids by direct injection-liquid chromatography-electrospray ionization-triple quadrupole tandem mass spectrometry and high resolution mass spectrometry: Occurrence and evolution in chlorine-treated water. *Analytical and Bioanalytical Chemistry* **411** (17), 3905–3917. https://doi.org/10.1007/s00216-019-01864-5.

Plewa, M. J., Simmons, J. E., Richardson, S. D. & Wagner, E. D. 2010 Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. *Environmental and Molecular Mutagenesis* **51** (8–9), 871–878. https://doi.org/10.1002/em.20585.

Postigo, C., Emiliano, P. & Valero, F. 2020 High-throughput and reliable determination of 13 haloacetic acids and dalapon in water and evaluation of control strategies. *Environmental Science: Water Research & Technology* **6** (9), 2499–2509. https://doi.org/10.1039/D0EW00296H.

Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R. & DeMarini, D. M. 2007 Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Research/Reviews in Mutation Research* **636** (1), 178–242. https://doi.org/10.1016/j.mrrev.2007.09.001.

Rook, J. J. 1974 Formation of haloforms during chlorination of natural waters. *Journal of Society for Water Treatment and Examination* **23** (Part 2), 234–243.

Samson, C. C. & Seidel, C. J. 2022 Assessing national HAA9 occurrence and impacts of a potential HAA9 regulation. *AWWA Water Science* **4** (6), e1312. https://doi.org/10.1002/aws2.1312.

Samson, C. C., Seidel, C. J., Summers, R. S. & Bartrand, T. 2017 Assessment of HAA9 occurrence and THM, HAA speciation in the United States. *Journal AWWA* **109** (7), E288–E301. https://doi.org/10.5942/jawwa.2017.109.0083.

Sawade, E., Fabris, R., Humpage, A. & Drikas, M. 2016 Effect of increasing bromide concentration on toxicity in treated drinking water. *Journal of Water and Health* **14** (2), 183–191. https://doi.org/10.2166/wh.2015.127.

SCA. 2021 *Estimation of Uncertainty of Measurement for Chemical and Physico-Chemical Determinands in Drinking Water*. Standing Committee of Analysts (SCA). Available from: https://secureservercdn.net/160.153.137.128/fkp.e54.myftpupload.com/wp-content/uploads/Blue_Book_Library/276.pdf (accessed 23 October 2023).

Simpson, K. L. & Hayes, K. P. 1998 Drinking water disinfection by-products: An Australian perspective. *Water Research* **32** (5), 1522–1528. https://doi.org/10.1016/S0043-1354(97)00341-2.

Takino, M., Daishima, S. & Yamaguchi, K. 2000 Determination of haloacetic acids in water by liquidchromatography-electrospray ionization-mass spectrometry using volatileion-pairing reagents. *Analyst* **125** (6), 1097–1102. https://doi.org/10.1039/B002576N.

Tung, H.-H., Unz, R. F. & Xie, Y. F. 2006 HAA removal by GAC adsorption. *Journal AWWA* **98** (6), 107–112. https://doi.org/10.1002/j.1551-8833.2006.tb07687.x.

Wagner, E. D. & Plewa, M. J. 2017 CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: An updated review. *Journal of Environmental Sciences* **58**, 64–76. https://doi.org/10.1016/j.jes.2017.04.021.

Wagner, H., Lin, R., Fisher, C., Bahten, K., Pohl, C. & Jack, R. 2017 *EPA METHOD 557.1: Determination of Haloacetic Acids in Drinking Water Using Two-Dimensional Ion Chromatography with Suppressed Conductivity Detection*. Available from: https://www.regulations.gov/document/EPA-HQ-OW-2017-0284-0030.

Weng, H., Wang, C., Ye, T., Xu, Z., Sun, H., Lin, H., Deng, W.-J., Wu, F. & Hong, H. 2022 Precursor characteristics of mono-HAAs during chlorination and cytotoxicity of mono-HAAs on HEK-293t cells. *Chemosphere* **301**, 134689. https://doi.org/10.1016/j.chemosphere.2022.134689.

Whitaker, H., Nieuwenhuijsen, M. J., Best, N., Fawell, J., Gowers, A. & Elliot, P. 2003 Description of trihalomethane levels in three UK water suppliers. *Journal of Exposure Science & Environmental Epidemiology* **13** (1), 17–23. https://doi.org/10.1038/sj.jea.7500252.

WHO. 2022 'Chemical Aspects', in *Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First and Second Addenda* [Internet]. World Health Organization. Available from: https://www.ncbi.nlm.nih.gov/books/NBK579467/ (accessed 25 January 2024).

Wu, F., Gabryelski, W. & Froese, K. 2002 Improved gas chromatography methods for micro-volume analysis of haloacetic acids in water and biological matrices. *Analyst* **127** (10), 1318–1323. https://doi.org/10.1039/B204574E.

Wu, S., Anumol, T., Gandhi, J. & Snyder, S. A. 2017 Analysis of haloacetic acids, bromate, and dalapon in natural waters by ion chromatography–tandem mass spectrometry. *Journal of Chromatography A* **1487**, 100–107. https://doi.org/10.1016/j.chroma.2017.01.006.

Xie, Y., Rashid, I., Zhou, H. & Gammie, L. 2002 Acidic methanol methylation for HAA analysis: Limitations and possible solutions. *Journal AWWA* **94** (11), 115–122. https://doi.org/10.1002/j.1551-8833.2002.tb10235.x.

Zaffiro, A., Zimmerman, M., Pepich, B., Slingsby, R., Jack, R., Pohl, C. & Munch, D. 2009 EPA METHOD 557: Determination of haloacetic acids, Bromate, and Dalapon in Drinking Water by ion chromatography electrospray ionization tandem mass spectrometry (IC-ESI-MS/MS), Version 1. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P1005OKO.txt.