ANALYSIS OF TEN HALOACETIC ACIDS IN CHLORINATED SURFACE WATER BY LC-MS-MS

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Introduction

Drinking water disinfection by-products (DBPs) are an unintended consequence of using disinfectants to kill harmful pathogens in drinking water. They are formed primarily by the reaction of disinfectants with natural organic matter, and bromide or iodide. DBPs have become a public health concern ever since they were discovered, due to their probable carcinogenicity, mutagenicity, as well as developmental, reproductive and hepatic toxicity[1,2]. Haloacetic acids (HAAs) are one of the most commonly detected classes of DBPs. The source of drinking water used by the majority of Canadians, Americans, and many others globally originates in forests. Forest fires are having an increasingly negative effect on source water and are posing a challenge to the design and operational response capacities of water quality treatment plants [3]. In this study we developed a simple and sensitive LC-MS-MS method that is suitable to analyze 10 haloacetic acids in chlorinated water. The method will be used to study the formation potential of DBPs of source waters to assess the effects of wildfires and forest management practices on water quality in Canada.

Materials and methods

HAA standards were purchased either from AccuStandard Inc. (New Haven, CT, USA) or Sigma-Aldrich Canada (Oakvilla, ON, Canada). All deuterated internal standards (ISTDs) were obtained from CDN isotopes Inc. (Pointe-Claire, QC, Canada). The working standards were prepared in methyl tert-butyl ether (MTBE) and stored frozen at -20°C.

The sample preparation procedure was as follows: 100 µL of ISTD mix (MCAA-d3, MBAA-d3 and DCAA-d2) and 1mL of water sample were added into 16X100mm culture tubes. 2 mL of MTBE, 200 µL of H_2SO_4 (1:1), 100 µL of $CuSO_4$ (1M) and 800 mg of $NaSO_4$ were then added to each tube. Tubes were capped and shaken for 15 min. on a mechanical shaker at high speed and then centrifuged for 10 min. at 4000rpm. The MTBE layer was transferred to a clean 16X100mm culture tube and 1 mL of D.I. water was added into each tube, then vortexed for 20 sec. The MTBE layer was evaporated under gentle N_2 flow. The final extract was transferred into a 2mL autosampler vial for LC-MS-MS analysis. The separation of HAAs was carried out on a 100x2.1 mm Atlantis® dC18 column (Waters, Mississauga, ON, Canada) using an Agilent 1260 HPLC system (Agilent technologies, Mississauga, ON, Canada) with mobile phase of 0.05% formic acid in D.I. water (A) and acetonitrile (B). Table 1 shows the time table for gradient elution. The injection volume was 20 µL and the column temperature was kept at 25 °C. The HAAs were detected using a 5500 Q-trap system (AB Sciex Concord, Ontario, Canada) operated in negative MRM mode. The source parameters were 20 (CUR), medium (CAD), -4500 (IS), 4500 (TEM), 50 (GAS1), 50 (GAS2) and -10 (EP). The MRM transitions, retention time and MS parameters for individual HAA are listed in table 2. A set of calibrators prepared by spiking known amounts of HAAs in the D.I. water was processed and analyzed along with samples to construct a calibration curve. The quantification and identification of each HAA was based on MRM transition(s) combined with the retention time using Multiquant® software from AB Sciex.

Table 1. Mobile phase gradient table

Time (min.)	Flow rate (µL/min.)	A%	B%
0	200	98	2
2.0	200	98	2
3.0	300	80	20
4.0	450	40	60
6.5	450	40	60

Time (min.)	Flow rate (µL/min.)	A%	B%
6.6	450	2	98
8.6	450	2	98
8.7	450	98	2
12.0	450	98	2

Table 2. MRM transitions, MS parameters and retention time for individual HAAs

Analyte Name	Analyte Acronym	R.T. (min)	DP	Quant. Ion			Qual. Ion		
				MRM 1	CE	CXP	MRM 2	CE	CXP
Monochloro-acetic acid	MCAA	2.46	-40	93/35	-19.8	-15.4	93/49	-20.1	-7.1
Dichloroace-tic acid	DCAA	2.45	-56	126.9/83	-13.0	-9.0	126.9/35	-32.0	-16.0
Bromochlo-roacetic acid	BCAA	2.63	-50	172.9/128.9	-15.0	-10.0	172.9/80.9	-26.2	-12.0
Monobromo-acetic acid	MBAA	2.81	-35	136.9/79	-14.0	-9.0	-	-	-
Dibromoace-tic acid	DBAA	2.98	-41	216.8/172.8	-14.6	-13.0	216.8/80.9	-32.0	-9.2
Iodoacetic acid	IAA	3.68	-32	184.9/126.9	-12.6	-11.0	-	-	-
Trichloro-acetic acid	TCAA	4.10	-31	160.9/117	-10.0	-13.0	160.9/35	-35.0	-15.0
Bromodi-chloroacetic acid	BDCAA	4.38	-35	206.8/80.9	-22.0	-9.3	206.8/162	-9.0	-20.0
Chlorodibro-moacetic acid	CDBAA	4.68	-40	206.8 /80.9	-21.0	-10.0	252.8/208.8	-9.2	-20.0
Tribromo-acetic acid	TBAA	5.18	-40	250.8/78.9	-50.0	-10.0	250.8/80.9	50.0	-8.2

Results and discussion:

Atlantis® dC18 column was chosen for the separation of the 10 HAAs. The chromatographic conditions were optimized for resolution and peak shape. Figure 1 shows the total ion chromatograms of the ten HAAs. It can be seen that baseline separation was achieved for most of the HAAs except for MCAA that co-eluted with DCAA, and MBAA that co-eluted with DBAA. The separation of these two pairs of HAAs was achieved on MS by using different MRM transitions. It is important to achieve LC column separation for the following three pairs of HAAs: BCAA/DBAA, BDCAA/CDBAA and CDBAA/TBAA. Usually for small molecules, the molecular ion is the dominant precursor ion formed in ESI source. However, for HAAs, dominant precursor ions formed in ESI can be either the molecular ion ($[M\text{-}H]^-$) or the decarboxylated ion ($[M\text{-}COOH]^-$), or both. The patterns were affected by the degree of halogen substitution and the mass spectrometry parameters used. In general, the relative signal intensity of $[M\text{–}COOH]^-$ increases as the halogen substitution increases [4]. Even though the molecular weight for BCAA, DBAA, CDBAA, BDCAA and TBAA is different from each other, they may form precursor ions with the same m/z and produce the same product ions. Table 3 lists precursor ions for BCAA, DBAA, BDCAA, CDBAA and TBAA found on AB Sciex 5500 Q-trap. It can be seen that ion (m/z 172.7) is the common precursor ion for BCAA and DBAA. Since both ions can be fragmented to Br^- (m/z 80.9) in the collision cell, ion 172.7/80.9 is the common MRM transition for both BCAA and DBAA. Because this MRM transition was used as a confirmation ion for BCAA, the presence of DBAA in the sample will interfere with the analysis of BCAA if they were not separated. Similarly, 206.8 is a common precursor ion for BDCAA and CDBAA, and 250.8 is a common precursor ion for CDBAA and TBAA. All three compounds can be fragmented to Br^- (m/z 80.9, 78.9). Thus 206.8/80.9 is the common MRM transition for BDCAA and CDBAA. 250.8/78.9 and 250.8/80.9 can be produced from both CDBAA and TBAA. MS is not able to differentiate those transitions from these three compounds if they were not separated on column. Hence, the LC conditions used in this method were optimized to achieve baseline separation for these three pairs of HAAs.

Fig 1 Total ion chromatogram of 10 HAAs

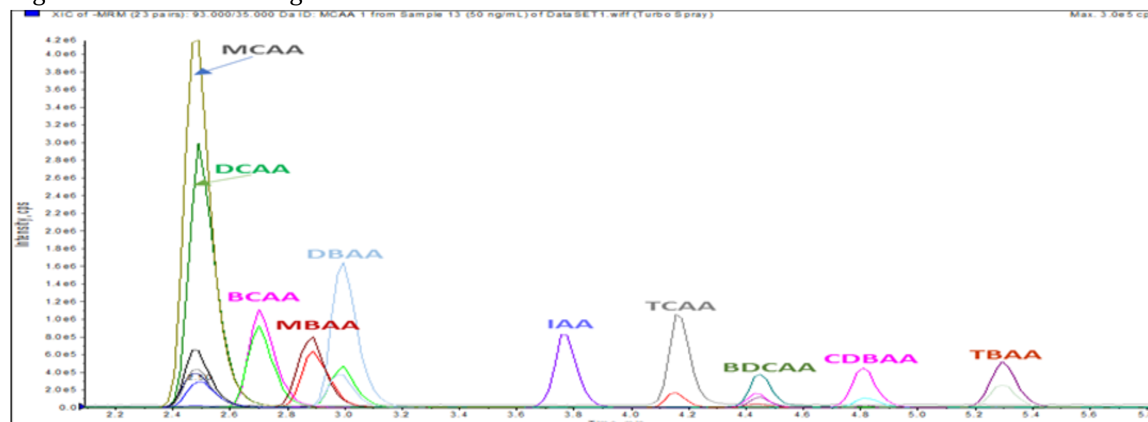


Table 3. Precursor ions formed in ESI for individual HAAs

Analyte Name	Precursor 1 $[M-H]^-$	Precursor 2 $[M-COOH]^-$
	m/z	m/z
BCAA	***172.9***	129.0
DBAA	216.8	***172.9***
BDCAA	***206.8***	162.8
CDBAA	***250.8***	***206.8***
TBAA	-	***250.8***

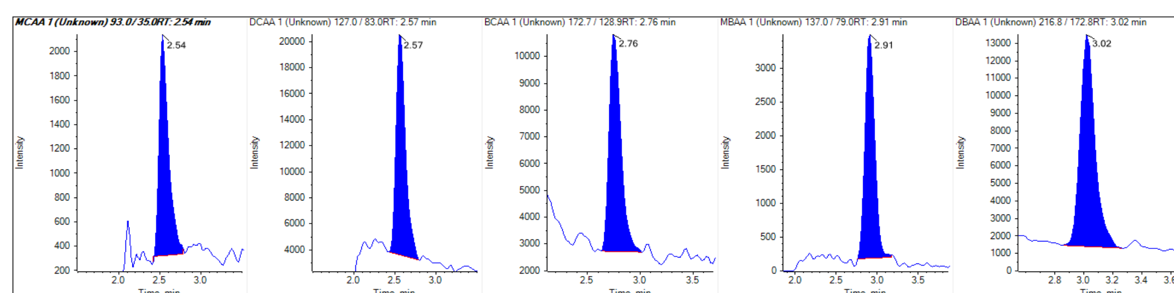
The presence of matrix ions such as chloride in the chlorinated surface water can interfere with the analysis of HAAs when the LC-MS-MS technique is used [5]. $NaCl_2^-$ (m/z 93), which can be further fragmented to Cl^- (m/z 35), was found in all chlorinated water samples and it interferes with the analysis of MCAA. Therefore, it is important to remove the matrix ion in chlorinated water. Liquid-Liquid extraction was chosen to clean up the samples. MTBE was used as the extraction solvent. After extraction, MTBE needs to be exchanged with a solvent that is compatible with LC-MS-MS analysis. Our first attempt was to dry down the final extract under a gentle stream of N_2 and reconstitute in D.I water. However, the recovery for TBAA was low (<50%) due to the loss of TBAA during drying down process. To improve the recovery for TBAA, solvent exchange was conducted by extracting HAAs back into the aqueous phase and then removing MTBE under N_2. With this technique, the loss of TBAA in the drying down process was minimized and a recovery of 85% was achieved.

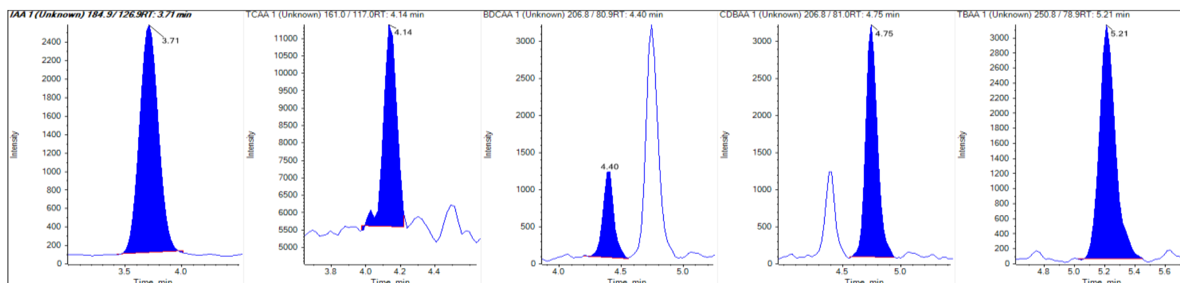
The method was validated for matrix effect, linearity, recovery, accuracy and precision. Method LOD and LOQ were assessed. No significant matrix effect was observed. All HAAs are linear up to 100 μg/L with R^2 greater than 0.99. Table 4 shows the validation results including method LOD, LOQ, recovery and precision (CV%). The accuracies were assessed at 20 μg/L and they ranged from 98% to 105%. The results demonstrated that good recovery, accuracy and precision were achieved with this method. The method LOD and LOQ were better than or comparable to published methods [6,7] using LC-MS-MS. Fig.2 shows the extracted ion chromatograms for each HAA at 2 μg/L.

Table 4. Method validation results

Analyte Name	LOD (μg/L)	LOQ (μg/L)	Recovery (%)			Precision (CV %)		
			2 (μg/L)	20 (μg/L)	80 (μg/L)	2 (μg/L)	20 (μg/L)	80 (μg/L)
MCAA	0.2	0.2	94.6	84.3	86.3	10.1	3.2	1.9
DCAA	0.1	0.2	94.3	84.1	87.5	5.6	1.7	2.0
BCAA	0.1	0.1	83.4	83.4	85.9	4.9	1.4	3.0
MBAA	0.1	0.1	86.4	86.4	87.0	3.7	3.4	3.6
DBAA	0.1	0.1	89.0	87.3	88.3	5.5	4.1	5.5
IAA	0.1	0.1	83.9	88.2	88.4	10.9	2.4	3.7
TCAA	0.5	0.5	91.6	84.7	90.9	10.1	1.8	3.4
BDCAA	0.1	0.5	83.8	83.4	89.7	11.4	3.5	4.6
CDBAA	0.1	0.1	83.7	85.9	92.0	6.0	2.5	3.2
TBAA	0.1	0.1	87.7	84.6	91.2	8.0	3.3	3.2

Fig 2. Extracted ion chromatograms of 10 HAAs at 2 μg/L.





The method was applied to determine HAAs in four water samples that were collected from water reservoirs in Calgary and chlorinated with 10 mg Cl_2/L and 20 mg Cl_2/L respectively. As shown in table 5, of the 10 target HAAs, only four were detected at low concentration. 20 mg Cl_2/L of disinfectants (free chlorine) produced more HAAs than those by 10 mg Cl_2/L.

Table 5. Water sample results (μg/L)

Analyte	A		B		C		D	
	10 mg Cl_2/L	20 mg Cl_2/L	10 mg Cl_2/L	20 mg Cl_2/L	10 mg Cl_2/L	20 mg Cl_2/L	10 mg Cl_2/L	20 mg Cl_2/L
MCAA	0.28	0.58	0.54	1.22	n.d.	0.20	0.46	0.74
DCAA	0.46	1.56	0.78	6.02	n.d.	0.68	1.56	2.06
BCAA	n.d.	0.20	n.d.	n.d.	n.d.	n.d.	n.d.	0.22
MBAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DBAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TCAA	<LOQ	<LOQ	1.02	1.68	n.d.	<LOQ	<LOQ	<LOQ
IAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDCAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CDBAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TBAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

In conclusion, we have developed a simple and sensitive method that is suitable for the analysis of ten haloacetic acids in chlorinated water.

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