Determination of Ten Haloacetic Acids in Drinking Water Using High-Performance and Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry

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Abstract

Haloacetic acids (HAAs) are a class of byproducts resulting from the reaction of chlorinated disinfectants with natural organic matter. These chemicals have been found in animal studies to possibly influence hepatic, reproductive, and developmental functions, and they may be mutagenic and carcinogenic. Because HAAs are hydrophilic and strongly acidic, it is a challenge to measure them at low levels. In this study, nine traditional HAAs and monoiodoacetic acid, an emerging disinfection byproduct, are analyzed in water directly. HAAs were separated on a BetaMax Acid column or a HILIC UPLC column, and they were detected by negative electrospray ionization-tandem mass spectrometry. Although the on-column limits of detection of HAAs were lower when using an HILIC UPLC column (0.08–2.73 µg/L) than when using a BetaMax Acid column (0.18 to 71.5 µg/L), to use an HILIC UPLC column, it was required to dissolve water samples in 90% acetonitrile before injection and result in sample dilution. BetaMax Acid column was found to be more suitable for the analysis of HAAs in drinking water because there was no need of sample preparation. Major species of HAAs, such as dichloroacetic acid and trichloroacetic acid, and other primary species (e.g., dibromoacetic acid, bromochloroacetic acid and bromodichloroacetic acid) can be detected using the BetaMax Acid column at concentrations higher than 1–3 µg/L.

Introduction

Disinfection byproducts (DBPs) may be produced through the reactions of disinfectants with natural organic matters in raw water. The most abundant DBPs resulting from chlorination are trihalomethanes, followed by haloacetic acids (HAAs), the next most abundant (1–3). There are nine major chlorinated and brominated acetic acids, including monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), and chlorodibromoacetic acid (CDBAA) (4). Humans are less exposed to trihalomethanes than to HAAs because they are more volatile than HAAs (boiling points ~60°C vs. boiling points ~180°C, respectively) and are usually removed during the boiling process of drinking water.

In animal studies, HAAs have been found to be toxic to liver and embryos and may be mutagenic, carcinogenic, and have reproductive effects in lab animals (5–9). The Integrated Risk Information System of the United States Environmental Protection Agency (U.S. EPA) has classified DCAA as a group B2 chemical (i.e., a probable human carcinogen) and TCAA as a group C compound (possible human carcinogen) (10,11). In addition, iodoacetic acids may be more toxic and mutagenic than chloro- and bromo-acetic acids (12).

Although the total concentrations of HAAs vary by seasons, water sources, and drinking water treatment plants, the most abundant species are usually TCAA and DCAA (13,14), making up about 80% of all HAA concentrations (13), followed by BDCAA and BCAA, which make up around 15% (13). The U.S. EPA has established a maximum contamination level of 60 µg/L for the total concentrations of five HAAs (HAA5: MCAA, DCAA, TCAA, MBAA, DBAA) (15); World Health Organization set up maximum contamination levels of DCAA and TCAA at 50 and 100 µg/L, respectively (3).

Most surveys report low-µg/L levels of HAAs in drinking water. In a study of 12 drinking water treatment plants in the US, Krasner et al. found the concentrations of the nine HAAs ranged 3–18 µg/L (16). In Canada, except at plants using chlorine as the disinfectant, concentrations were found to be lower than 50 µg/L for most HAAs (17). Analyzing tap water from 10 locations in Taiwan, Hsu found the individual concentrations of six HAAs to range from 1–13 µg/L and total concentrations to range from 5–33 µg/L (18).

The standard method of analyzing HAAs in drinking water is the U.S. EPA Method 552 (19). Because of the strongly acidic characters (pKa range 0.63–2.90) of HAAs (20,21), samples are acidified to reduce the dissociation of HAAs, which are extracted using methyl-*t*-butyl ether. The extracts are esterified with methanol to enhance the volatility of the analytes and then are concentrated under gentle nitrogen flow. The residues are then analyzed using gas chromatography–electron capture detection (GC–ECD). Although highly sensitive (0.012-0.17 µg/L), this method is time-consuming and labor-intensive. Another limita-

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tion of this method is that the strong acidity of the final solution shortens GC column life and HAA derivatives may undergo dechlorination and thermodegradation in the GC injection port (22,23).

Because there is no need for derivatization, liquid chromatography may be used to analyze HAAs. Capillary electrophoresis can effectively separate ionic HAAs; because of limited injection capacity and a less-sensitive UV detection, it requires a high enrichment factor to detect low µg/L levels of HAAs (20,24). Ion chromatography (IC) and post-column suppressor have been coupled with electrospray-mass spectrometry (ESI-MS) and has been found to have sub-µg/L sensitivity, but to date, this method has only been used with DCAA/TCAA or HAA5 (25–27).

Reversed-phase liquid chromatography (RP-LC) is usually not able to retain HAAs; although acidic buffer or ion-pairing reagents have been used to increase the retention, the LC conditions may somewhat suppress the ionization of HAAs on ESI, and the methods require pre-enrichment or a large-volume injection of water (28–30). Recently, there has been increased use of hydrophilic interaction liquid chromatography (HILIC) to retain polar molecules (31–33). While it is a type of normal-phase chromatography, it uses water and water-miscible solvents as mobile phases (33,34). In one previous study using this method, Dixon et al. dried water samples (500 µL) under low vacuum and then reconstituted the residue with 100 µL of acetonitrile–water (60:40, v/v). They injected 10 µL of the sample to analyze DCAA with HILIC–ESI-MS–MS, and this method enabled them to detect at levels as low as 1 µg/L (34). There are reports of the use of HILIC on DCAA (33,34) but not for other HAAs.

In this study, we used negative ESI-MS–MS in the development of direct methods of analyzing HAAs in drinking water with minimal sample preparation. We compared the simultaneous separation of 10 HAAs, including monoiodoacetic acid (MIAA), an emerging relatively more toxic DBP, using a traditional reversed-phase column with their separation using an ultra-performance liquid chromatography (UPLC) HILIC column. We found that although the limit of detection (LOD) of several HAAs were not as sensitive as the standard method, we did not need to go through sample preparation, extraction, derivatization, or concentration to perform our measurements. This study also discusses issues related to matrix effect and calibration linearity.

Experiment

Chemicals and reagents

Individual chemicals of HAAs were obtained from Chem Service (West Chester, PA) except for MCAA (Sigma, St. Louis, MO) and MIAA (Fluka, Buchs, Switzerland). Solvents, including methanol, acetone, heptane, acetonitrile, and dichloromethane, were all HPLC grade and obtained from J.T. Baker (Phillipsburg, NJ).

Instrumental conditions

Samples of drinking water were analyzed with a BetaMax Acid column (2.1 × 250 mm, 5 µm; Thermo Hypersil-Keystone, Bellefonte, PA) or an Acquity UPLC BEH HILIC column (2.1 × 100 mm, 1.7 µm; Waters, Milford, MA) using a Waters Acquity UPLC system coupled with a Waters Quattro Premier XE triple-quadrupole mass spectrometer and negative electrospray ionization. The chromatographic temperature of BetaMax Acid column was set at 40°C and mobile phases were (A) aqueous 3.5 mM acetic acid–20 mM ammonium acetate (pH 5.3) and (B) acetonitrile. The flow rate was 0.2 mL/min. Gradient was increased from 10% to 73% of solvent B over 7 min, kept constant for 5 min, and then increased to 100% B over another 1 min, and then kept constant for 0.5 min. The column was flushed at 100% B for 2.5 min at a flow rate of 0.35 mL/min, and the gradient was decreased back to its initial composition in 2 min and re-equilibrated for another 2 min before the next injection. The total time of the run was 20 min, and the injection volume was 50 µL.

HILIC UPLC separation was run at 25°C at a flow rate of 0.5 mL/min. The injection volume was 30 µL, and the sample composition was in 90% acetonitrile–10% water. The mobile phase was composed of (A) aqueous 5 mM formic acid–10 mM ammonium formate (pH 4.1) and (B) acetonitrile. Gradient was decreased from 90% to 80% of solvent B in 2 min. The gradient was returned to its initial condition for a 3-min re-equilibrium before the next injection. The total run time was 5 min. The parameters of MS were optimized for the 10 analytes, and data were acquired using multiple-reaction monitoring (MRM) using three separate segments to increase data points across peaks. 2-bromobutyric acid (2-BBA) was used as the surrogate chemical. The dwell time for each analyte was 0.2–0.3 s for the BetaMax Acid column, and 0.075–0.085 s for the HILIC UPLC column, respectively (Table I). The capillary voltage was 3 kV. The source temperature and desolvation temperature were 130°C and 400°C, respectively. Nitrogen was used for the desolvation gas (900 L/h) and cone gas (50 L/h). Argon was used as the collision gas at a pressure of 3.8×10^{-3} mbar.

Standards, calibrations, and QA and QC

Stock solutions of HAAs and 2-BBA were prepared in deionized/distilled water obtained from a Milli-Q unit (Millipore, Bedford, MA) at 1.0 mg/mL and were stored at 4°C. A mixture of the 10 HAAs was made at 0.1 mg/mL and solutions of mixed HAAs in lower concentrations were prepared by a series of dilutions with the Milli-Q water; 2-BBA was added separately within the 2% of the total volume. Before the instrumental analysis, standards for HILIC UPLC were diluted in water with 9-fold acetonitrile (water–ACN, 1:10, v/v). Calibration curves were built up using at least five different concentrations, square of correlation coefficients being larger than 0.98.

After use, all glassware was washed with detergent and was flushed thoroughly with both tap water and Milli-Q water, and rinsed with acetone, heptane, dichloromethane and methanol. For each batch of analysis, there was a Milli-Q water blank and a blank spiked with 2-BBA. We observed no experimental contamination. Data were analyzed using Waters MassLynx V 4.1 and Microsoft Excel 2003. The LOD and limit of quantitation (LOQ) were defined as signal/noise (S/N) ratios at 3 and 10, respectively.

Concentration of tap water

Tap water was filtered through a 47-mm PTFE filter (pore size 0.22 µm; Millipore). For 40-fold enrichment, 10 mL of filtered

water was concentrated to 250 µL using a SpeedVac (SPD 1010, Thermo Savant, Holbrook, NY) under 60°C and –7 torr (–0.01 atm). For 400-fold enrichment, 200 mL of filtered water was concentrated to less than 5 mL using a rotary evaporator (Büchi, Flawil, Switzerland) and was further concentrated to 500 µL using a SpeedVac. The concentrated water was filtered again with a 0.22-µm PTFE syringe filter. For HILIC UPLC analysis, the concentrated water was diluted for 10 times with acetonitrile before injection.

Evaluation of matrix effects

Matrix effects were evaluated by comparing the peak area of post-spiked tap-water samples after concentration (A') with that of the same amount of standards (A_s). The peak areas of endogenous HAAs in the non-spiked tap water after concentration (A_0) were deducted. The ion suppression (%) was calculated as $1 – (A' – A_0)/A_s \times 100\%$. The higher the percentage, the more significant the ion suppression.

Results and Discussion

LC–MS–MS conditions

We observed three types of precursor HAA ions reported by previous studies: $[M–H]^-$ (pseudomolecular ion), $[M–COOH]^-$ (decarboxylated ion) or $[2M–H]^-$ (dimer ion), which were similar to previous reports (30,35). Their relative signal intensities varied according to infusion concentrations and ESI parameters. For example, dimer ions were the major precursor ions of MCAA, DCAA, and TCAA at high infusion concentrations (e.g., 100 ng/µL), as reported by Roehl et al. (35); however, when HAAs were injected onto LC at pg/µL levels, $[M–H]^-$ became predominant. The dimer ions did not prove to be good candidates for MRM precursor ions as the signal intensities of $[M–H]^- \rightarrow [M–COOH]^-$ were still higher than those of dimer ions $\rightarrow [M–H]^-$, even when the dimer ions were principal precursors.

The degree of halogen substitution also influenced the patterns of HAA ions formed in ESI. As relative signal intensity of $[M–COOH]^-$ increased, so to did halogen substitution, especially for BCAA, BDCAA, CDBAA, and TBAA. However, except for TBAA, deprotonated ions produced more intense signals than decarboxylated ions for MRM, a finding somewhat different from those studies using selected ion monitoring (SIM). Roehl et al. proposed that $[M–COOH]^-$ was more favored in ESI than $[M–H]^-$ for more heavily substituted HAAs (35); therefore, they chose $[M–COOH]^-$ when doing SIM for CDBAA and TBAA, and $[M–H]^-$ for other lighter HAAs, as the precursor ions we chose in our study. Takino and colleagues, on the other hand, reported that $[M–COOH]^-$ is the most intense ion for TCAA, BDCAA, CDBAA and TBAA, and they did not observe either $[M–H]^-$ or $[2M–H]^-$ in their study of these four HAAs (29). Like Takino et al., Loos and Barceló used $[M–COOH]^-$ as base peaks for their SIM of TCAA, BDCAA, CDBAA, and TBAA (30). The differences in the relative intensities of base ions in ESI may have resulted from differences in the designs of the ESI probes and differences in operating conditions.

$[M–H]^-$ were the most suitable precursor ions for monohaloacetic acids, MCAA, MBAA, and MIAA (*m/z* 92.9, 136.8, and 184.8, respectively). Eleven volts was the best collision energy need to generate their product ions, $[Cl]^-$, $[Br]^-$, and $[I]^-$ (Table I). Although MCAA and MBAA were able to produce the product ions of [M-COOH]$^-$, their signals were lower than those of halogen ions.

$[M–H]^-$ were the best precursor ions of DCAA and TCAA (*m/z* 126.8 and 160.8, respectively); $[M–COOH]^-$ were the best product ions (m/z 83 and 116.8, respectively), and the best colli-

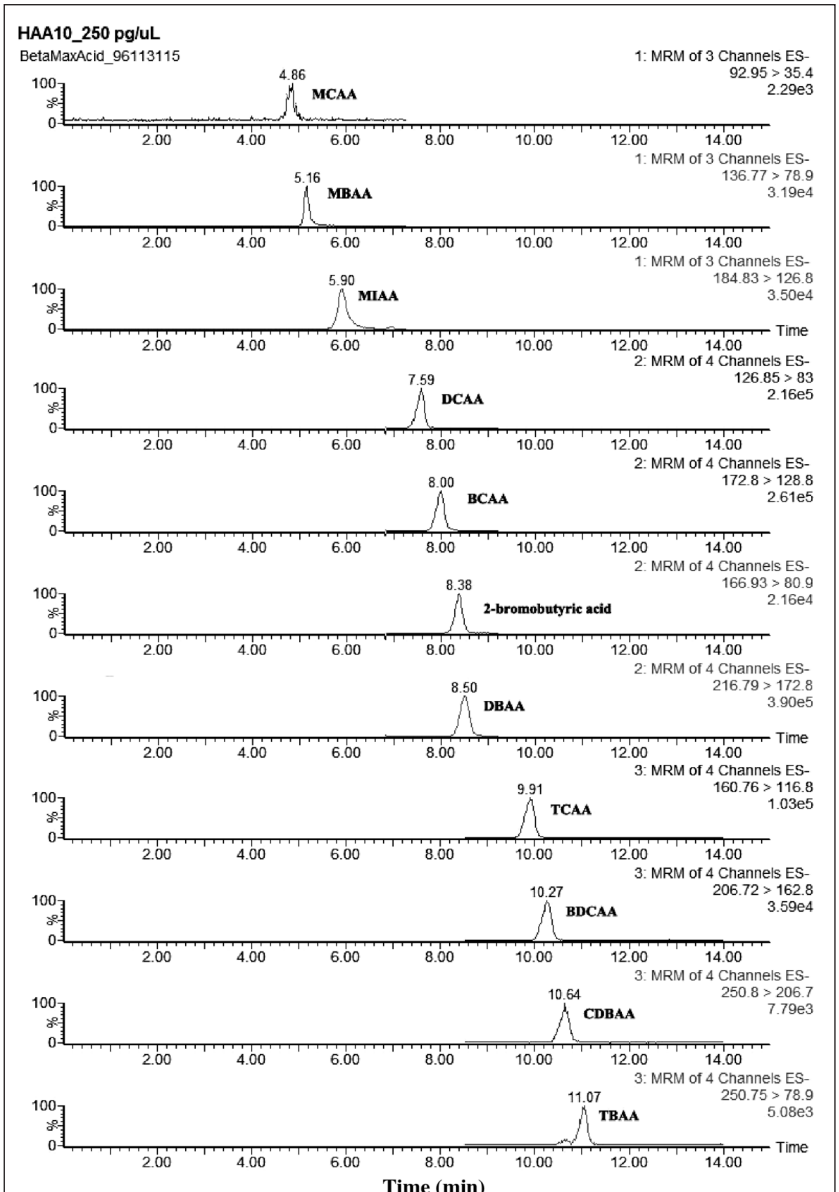

Figure 1. The chromatogram of HAAs on BetaMax Acid column (individual HAA concentration was 250 pg/µL, injection 50 µL).

Table I. The Optimized Parameters for MRM

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (sec) BetaMax	Dwell time (sec) HILIC	Cone voltage (V)	Collision energy (V)
MCAA	92.9	35.4	0.25	0.080	20	11
MBAA	136.8	78.9	0.30	0.080	20	11
MIAA	184.8	126.8	0.30	0.080	11	11
DCAA	126.8	83.0	0.25	0.075	20	11
DBAA	216.8	172.8	0.20	0.075	20	11
TCAA	160.8	116.8	0.25	0.085	15	7
TBAA	250.8	78.9	0.20	0.085	25	13
BCAA	172.8	128.8	0.30	0.075	20	9
BDCAA	206.7	162.8	0.25	0.085	20	7
CDBAA	250.8	206.7	0.25	0.085	15	7
2-BBA	166.9	80.9	0.25	0.080	15	9

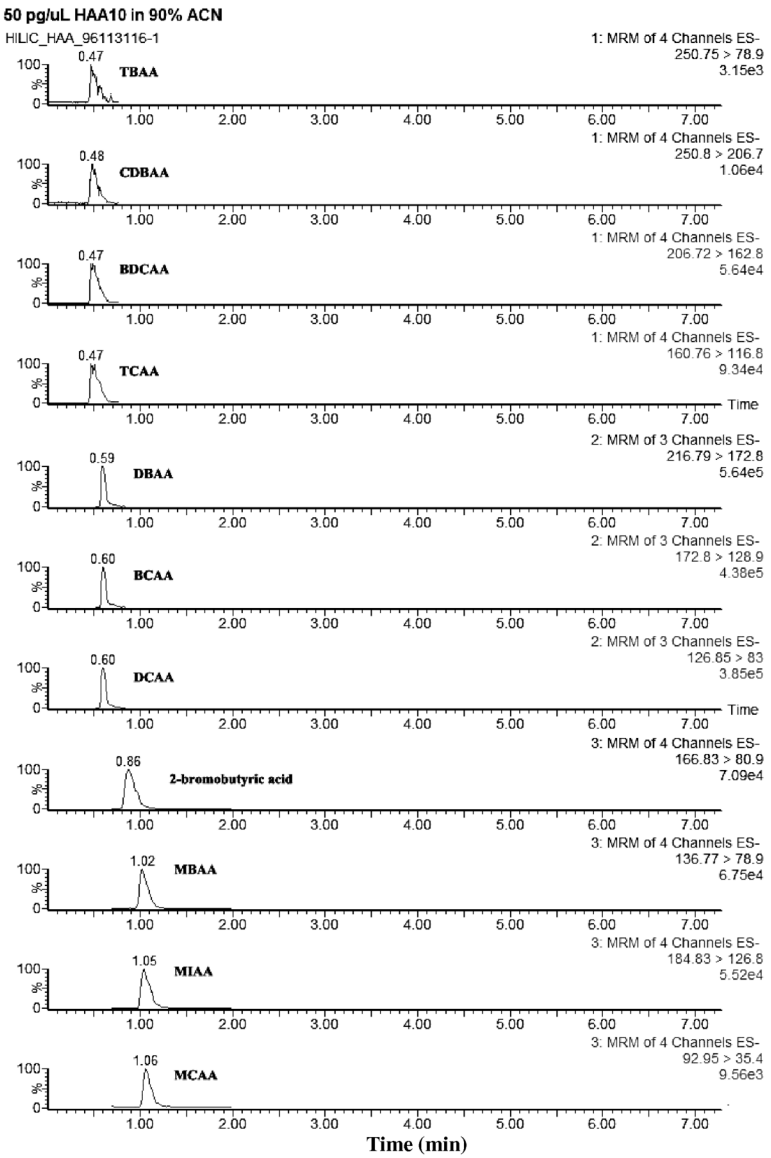

Figure 2. The chromatogram of HAAs on HILIC UPLC column (individual HAA concentration was 50 pg/µL, injection 30 µL).

sion energies were 11 V and 7 V, respectively (Table I). Although DCAA and TCAA also produced the product ion $[Cl]^-$, its intensity was not as strong as that of $[M\text{-}COOH]^-$.

Bromoacetic acids, including MBAA, DBAA, BCAA, BDCAA, CDBAA, and TBAA, were all able to produce the product ion of $[Br]^-$. The precursor ion of TBAA was $[M–COOH]^-$. The product ion of $[Br]^-$ was produced at a collision energy of 13 V (Table I). The most suitable precursor ions of DBAA, BCAA, BDCAA and CDBAA were $[M–H]^-$, which were *m/z* 216.8, 172.8, 206.7, and 250.8, respectively; the best product ions were $[M–COOH]^-$ rather than $[Br]^-$, which were *m/z* 172.8, 128.8, 162.8, and 206.7, respectively, and the best collision energies were 11 V, 9 V, 7 V, and 7 V, respectively (Table I).

Ammonium acetate buffer was needed to retain HAAs on the BetaMax Acid column. HAAs were not retained at all when only Milli-Q water was used as the aqueous mobile phase. Except for CDBAA, 20 mM ammonium acetate (pH 6.3) gave better peak shapes and signals than 20 mM ammonium formate (pH 6.65). We acidified the solution with acetic acid and found that 3.5 mM acetic acid–20 mM ammonium acetate (pH 5.3) increased the retention of HAAs (capacity factor k' = 2.9–7.9; Figure 1) and enhanced their signals because they were eluted out at higher organic (ACN) portions for a better spray at ESI. Further acidification elongated the retention time but suppressed the signal. An initial mobile phase composition of 30% organic retained the HAAs and produced a signal intensity two to 10 times that produced by 10% organic; nevertheless, this caused the peak overlapping of CDBAA and TBAA. Their precursor ions $[CDBAA–H]^-$ and $[TBAA–COOH]^-$ were both *m/z* 251, and both can produce a product ion of $[Br]^-$, which CDBAA may interfere with the detection of TBAA. To prevent this interference, the initial organic fraction was kept at 10%.

The best temperature for the BetaMax Acid column was 40°C and the maximum injection volume was 50 µL. A lower temperature at 25°C improved the separation of HAAs but increase the time by 10 min (to a total of 30 min). An increase to 60°C did not improve peak shapes and signals. In addition, when the injection volume was increased to 75 µL or 100 µL of water, the peaks started to broaden, distort, and tail. There was no increase in HAA signals.

BetaMax Acid, which is a C12 column with polar-embedded groups, gave a much better retention on HAAs than a usual C18 column. We have tried to optimize the separation of HAAs on a BetaBasic C18 column (2.1 × 150 mm, 3 µm; Thermo Hypersil-Keystone), but found that most HAA peaks began to tail or broaden seriously, even in double peaks.

For HILIC UPLC, the optimal composition of initial mobile phase and injection solution was 90% acetonitrile–10% aqueous phase (v/v). A

direct injection of water samples caused early elution and tailing of peaks. A percentage of acetonitrile greater than 90% deteriorated peak shapes. The optimal composition of aqueous buffer was 5 mM formic acid–10 mM ammonium formate (pH 4.1) and the capacity factors ranged from 1.35–4.30 (Figure 2). Different from the BetaMax acid column, 10 mM of ammonium formate buffer gave better signals of HAAs than ammonium acetate at the same concentration, and 20 mM ammonium formate increased the retention of HAAs but suppressed their signals. The addition of 5 mM formic acid further improved the retention but did not influence the signal intensity.

The best temperature and the maximum injection volume for the HILIC UPLC columns were 25°C and 30 µL, respectively. A higher temperature at 40°C did not improve the peak shape or the separation. Moreover, an increase of the injection volume larger than 30 µL broadened peaks and did not increase the signals of HAAs.

The HILIC UPLC shortened the run time to one fourth of that using a BetaMax Acid column. Although many peaks of HAAs overlapped or co-eluted, they could all be qualified and quantified using MRM except for the product ion of $[Br]^-$ from CDBAA, which may interfere with the identification of TBAA. This effect could be neglected when the concentration of CDBAA in the injected solution was lower than 50 pg/µL.

Sensitivity

The on-column LOD of HAAs using the BetaMax Acid column ranged from 0.18 to 71.5 µg/L (9.2–3580 pg injection; Table III). DCAA, DBAA, TCAA, and BCAA had low LODs ranging from 0.18 to 0.39 µg/L (9–19 pg injection); MBAA, MIAA, and BDCAA had LODs around 1.1–3.2 µg/L (53–161 pg injection); the LODs of MCAA, TBAA, and CDBAA were inferior to others, which were at 6.6–71 µg/L (330–3580 pg injection).

Without pre-concentration, our method using the BetaMax Acid column was able to directly analyze major HAAs in drinking water. The LOQs for DCAA, DBAA, TCAA, BCAA, and BDCAA ranged 1.0 to 3.5 µg/L, which were sensitive enough to be used with tap water samples (Table III). The LOQs of MCAA, MBAA, MIAA, TBAA, and CDBAA ranged from 11.5–222 µg/L; a pre-concentration step would be needed to measure these HAAs in tap water. On the other hand, it should be mentioned that our LC–MS–MS method had a similar or better sensitivity than the existing IC–ESI-MS–MS or LC–ESI-MS–MS methods excluding their pre-enrichment process. Bruzzoniti et al. enriched samples for 62.5 times, and the LODs of HAA5 were from 1.5 to 10 µg/L (26). Liu et al. reported LODs of DCAA and TCAA to be 0.053 and 0.46 µg/L, respectively (27). Takino and colleagues, using a large-volume injection (500 µL), reached the minimum quantitation limit (MQL, 10 times the standard deviation) of nine HAAs at 0.024–0.12 µg/L (29). Dixon et al., concentrating samples at five times, found an LOQ of DCAA to be 5 µg/L (34). Loos and Barceló concentrated samples for 166.6 times and found the LOQs of the nine HAAs to range from 0.1 to 2.4 µg/L (30).

The HILIC UPLC column had better on-column sensitivity than the BetaMax Acid column. The on-column LOD and LOQ of HAAs using the HILIC UPLC column were 0.08–2.7 µg/L (2.3–82 pg injection) and 0.39–9.8 µg/L (11.7–250 pg injection), respectively (Table II). Most HAAs had good LODs and LOQs at 0.08–0.57 µg/L (2.3–17 pg injection) and 0.39–2.6 µg/L (12–78 pg injection), respectively. Similar to that of BetaMax Acid column, LODs and LOQs of MCAA, TBAA, and CDBAA using the HILIC column were inferior to those of other HAAs, which ranged from 1.32 to 2.73 µg/L (40–82 pg injection) and 5.6–9.8 µg/L (170–290 pg injection). Except for BCAA, LOD of all other nine HAAs using the HILIC UPLC were 2.5 to 26 times better than when the BetaMax Acid column was used. HILIC UPLC combined the benefits of sharper peaks by UPLC and a higher organic portion of eluted mobile phase by HILIC (better ionization efficiency in ESI). Because filtered water samples could not be injected onto HILIC UPLC directly and needed a 10-fold dilution with acetonitrile, a pre-concentration step using an organic solvent (e.g., liquid–liquid extraction or solid-phase extraction) was required to take full advantage of HILIC UPLC.

HAAs with a lower pKa were easier to dissociate and formed ions using ESI, but we observed no correlation between the signal intensity of HAAs and their pKa.

Table II. On-Column LOD* and LOQ† (mean ± SD, *n* = 7)

	BetaMax Acid column		HILIC UPLC column	
HAAs	LOD (µg/L)	LOQ (µg/L)	LOD (µg/L)	LOQ (µg/L)
MCAA	71.5 ± 13.6	222 ± 128	2.73 ± 0.69	9.78 ± 1.80
MBAA	3.09 ± 0.41	11.7 ± 5.5	0.57 ± 0.08	2.61 ± 0.19
MIAA	3.21 ± 0.35	11.5 ± 4.3	0.39 ± 0.09	2.01 ± 0.68
DCAA	0.39 ± 0.11	1.58 ± 0.83	0.12 ± 0.03	0.39 ± 0.13
DBAA	0.20 ± 0.03	1.58 ± 1.04	0.08 ± 0.02	0.51 ± 0.47
TCAA	0.36 ± 0.04	1.84 ± 0.45	0.21 ± 0.02	0.58 ± 0.14
TBAA	18.5 ± 2.0	95.8 ± 38.2	1.95 ± 0.69	8.33 ± 1.53
BCAA	0.18 ± 0.02	1.03 ± 0.54	0.41 ± 0.11	1.17 ± 0.29
BDCAA	1.06 ± 0.21	3.47 ± 0.86	0.30 ± 0.07	1.10 ± 0.14
CDBAA	6.59 ± 1.62	26.7 ± 5.2	1.32 ± 0.56	5.63 ± 0.32

* LOD = limit of detection (S/N = 3).
† LOQ = limit of quatitation (S/N = 10).

Table III. Concentration Recoveries of HAAs from Spiked Water

	Recoveries % [mean ± SD (RSD%), *n* = 4]	
HAAs	40-fold concentration*	400-fold concentration†
MCAA	71.0 ± 15.0 (21%)	102 ± 25 (25%)
MBAA	69.7 ± 3.2 (8.9%)	86.2 ± 7.7 (8.9%)
MIAA	114 ± 3.1 (2.7%)	93.6 ± 5.0 (5.4%)
DCAA	99.0 ± 3.2 (3.2%)	102 ± 6.1 (6.0%)
DBAA	96.5 ± 2.4 (2.4%)	101 ± 1.8 (1.8%)
TCAA	105 ± 1.8 (1.7%)	101 ± 4.5 (4.4%)
TBAA	108 ± 10 (8.9%)	93.0 ± 11.4 (12%)
BCAA	98.5 ± 1.3 (1.3%)	98.5 ± 3.9 (3.9%)
BDCAA	97.6 ± 1.7 (1.8%)	92.9 ± 7.8 (8.4%)
CDBAA	92.1 ± 3.0 (3.3%)	92.5 ± 10.1 (7.9%)

* Post-spiked levels after concentration were 50 µg/L except for MCAA (250 µg/L).
† Post-spiked levels after concentration were 125 µg/L.

Evaluation of concentration recoveries and matrix effects

To improve the sensitivity, we concentrated filtered water 40 or 400 times. The recoveries of HAAs were good and reproducible during the concentration steps. The recoveries of HAAs in Milli-Q water after 40-fold concentrations were 70–114% with small variations (RSD 1.3 to 8.9%, four duplicates) except for MCAA (RSD 21%) (Table III). Similarly, the recoveries after 400-fold concentrations were 86–102% (RSD 1.8–12%, except for MCAA at 25%) (Table III). Because HAAs were highly dissociated in the Milli-Q water and in low volatility, there was no significant loss of HAAs during the concentration steps.

Although the direct concentration approaches gave good recoveries, we did not use them to analyze HAAs in tap water samples because we observed significant ion suppression in concentrated tap water with both the BetaMax acid column and HILIC UPLC column. For BetaMax acid column, the signals of HAAs were suppressed 13–89% (Table IV). Those HAAs with better retention were less susceptible to matrix effects. For example, the ion suppression % of TBAA, BDCAA, CDBAA (k' 7.2–7.9) ranged between 13% and 36%. On the other hand, MCAA and MBAA, whose k' were smaller than 3.1, were subject to significant ion suppression. MCAA was not detected at the 400-fold concentrated tap water samples. Ion suppression was more significant when using the HILIC UPLC column than when using the BetaMax Acid column, which had signals of HAAs being suppressed between 53% and 89% (Table IV). Similar to the BetaMax Acid column, the signals of later eluted MCAA, MBAA and MIAA (k' 4.1–4.3) were less suppressed than other HAAs (k' 1.35–2.0). Because the degree of ion suppression of 2-BBA seemed similar to that of HAAs on HILIC UPLC column, we tested whether it could be a good internal standard and cancel out the matrix effect. The response factor ratios of MCAA and MBAA to 2-BBA were 19% and 23% lower than those of their standards, respectively, but others still ranged between

Table IV. Ion Suppression of HAA in Tap Water After Concentration

	Ion suppression % [mean ± SD (RSD%), n = 4]		
	BetaMax Acid column		HILIC UPLC column
HAAs	40-fold concentration*	400-fold concentration†	40-fold concentration‡
MCAA	85.4 ± 4.2 (5.0%)	(< LOD)	65.6 ± 7.9 (12%)
MBAA	77.3 ± 4.1 (5.3%)	88.6 ± 5.6 (%)	70.5 ± 6.8 (9.6%)
MIAA	37.5 ± 5.8 (15%)	61.4 ± 4.0 (%)	53.4 ± 7.0 (13%)
DCAA	60.3 ± 7.2 (12%)	64.7 ± 7.5 (%)	84.2 ± 3.0 (3.5%)
DBAA	73.6 ± 1.8 (2.5%)	69.2 ± 3.8 (%)	85.5 ± 2.4 (2.8%)
TCAA	57.9 ± 3.4 (5.8%)	47.0 ± 5.9 (%)	87.9 ± 2.6 (3.0%)
TBAA	22.1 ± 4.3 (19%)	36.5 ± 2.1 (%)	89.2 ± 3.8 (4.2%)
BCAA	56.9 ± 1.3 (2.3%)	60.5 ± 4.7 (%)	85.7 ± 2.2 (2.5%)
BDCAA	19.1 ± 2.7 (14%)	30.7 ± 0.4 (%)	88.7 ± 1.4 (1.6%)
CDBAA	33.7 ± 5.4 (16%)	12.6 ± 0.7 (%)	83.2 ± 1.6 (2.0%)
2-BBA§	24.4 ± 3.0 (12%)	41.3 ± 4.8 (%)	69.6 ± 1.9 (2.8%)

* Post-spiked levels after concentration were 50 µg/L except for MCAA (500 µg/L), TBAA (500 µg/L) and CDBAA (100 µg/L).
† Post-spiked levels after concentration were 500 µg/L.
‡ Tap water was concentrated for 400 times, then was diluted with acetonitrile for 10 times, then was spiked to form 50 µg/L solutions.
§ Post-spiked levels were kept at 10 µg/L.

Table V. Linearity of Calibration Curves

	BetaMax Acid column		HILIC UPLC column	
HAAs	Linear range (pg/µL)*	r^2	Linear range (pg/µL)†	r^2
MCAA	190–000	0.995	4.25–50	0.979
MBAA	10–1000	0.997	1.37–50	0.994
MIAA	5.0–1000	0.997	0.80–50	0.999
DCAA	3.0–50	0.988	0.30–50	0.998
DBAA	1.25–50	0.990	0.15–50	0.996
TCAA	1.8–100	0.998	0.37–50	0.989
TBAA	25–500	0.999	10–500	0.999
BCAA	0.75–25	0.996	0.12–50	0.999
BDCAA	3.7–50	0.994	0.50–50	0.997
CDBAA	5–100	0.980	4.5–100	0.991

* Injection volume 50 µL.
† Injection volume 30 µL.

Table VI. Intra-Day Accuracy and Precision of the Method using BetaMax Acid Column (*n* = 4)

HAAs	Spiked sample concentration (µg/L)	Measured concentration (µg/L) (mean ± SD)	RSD (%)*	Error (%)†
MCAA	375	362 ± 11	3.14	–3.47
	750	776 ± 46	5.95	3.47
	1500	1456 ± 24	1.66	–2.93
MBAA	20	21.6 ± 2.2	10.1	8.00
	40	41.2 ± 2.9	7.07	3.00
	80	75.4 ± 4.2	5.53	–5.75
MIAA	15	14.9 ± 0.5	3.43	–0.67
	30	29.5 ± 1.5	4.94	–1.67
	60	60.4 ± 0.9	1.48	0.67
DCAA	2.5	2.6 ± 0.2	9.30	3.20
	5.0	4.9 ± 0.6	12.6	–2.80
	10	8.9 ± 0.9	9.72	–11.1
DBAA	2.5	2.6 ± 0.1	4.98	6.00
	5.0	5.2 ± 0.5	10.7	3.80
	10	9.6 ± 0.4	4.44	–3.60
TCAA	3.6	3.8 ± 0.4	9.81	6.74
	7.1	7.5 ± 0.4	5.75	5.61
	14.3	15.0 ± 1.0	6.60	4.90
TBAA	125	139 ± 20	14.5	11.2
	250	295 ± 25	8.60	18.0
	500	520 ± 12	2.21	4.00
BCAA	1.5	1.6 ± 0.09	5.60	7.33
	3.0	3.0 ± 0.3	8.77	–1.33
	6.0	5.6 ± 0.4	6.81	–6.83
BDCAA	14.9	15.2 ± 2.0	12.9	2.01
	18.6	18.5 ± 0.8	4.48	–0.54
	29.8	26.4 ± 2.9	11.1	–11.4
CDBAA	12.5	15.1 ± 2.6	17.3	20.8
	25	26.7 ± 2.6	9.67	6.80
	50	53.8 ± 6.2	11.5	7.60

* RSD (%) = standard deviation/mean × 100.
† Error (%) = (measured concentration – spiked concentration)/spiked concentration × 100.

42% and 66% lower. Consequently, 2-BBA could not correct the influence of matrix effects on quantitation. Although tap water is a relatively clean matrix, the ions (e.g. Cl^-, SO_4^{2-}) and organic molecules inside could be enriched after the pre-concentration steps and influenced ESI efficiency and quantitation (27). Matrix effect is a critical issue when using LC–MS(–MS) for quantitation; however, the investigations on matrix effects are very rare on the analysis of HAAs in drinking water or tap water. Takino et al. reported a small matrix effect when a large-volume injection (500 µg/L) were used (29).

Method validation

The linear range of calibration of HAAs on ESI-MS–MS was two orders of magnitude or less, and was compound-dependent (Table V). For example, the response factor of DACC began to decrease at 100 pg/µL (5000 pg injection at BetaMax Acid column). The square of correlation coefficient (r^2) was 0.988 when the upper limit of the calibration was at 50 pg/µL but dropped to 0.981 when that calibration was extended to 100 pg/µL. Dixon et al. reported a linearity of 5–500 pg/µL (50–5000 pg injection) at $r^2 > 0.99$; however, the average calculated response of DCAA at 500 pg/µL was 462 ± 12 pg/µL, which revealed a saturation phenomena on ESI (34). The injection amount used in that study was the same as ours, 5000 pg. Delinsky et al. reported a linear range of 10–250 pg/µL (100–2500 pg injection) of DCAA (33). The upper range of the amounts they injected were the same as our highest linear calibration point (2500 pg). Liu et al. presented a linear range of DCAA at two orders of magnitude (27), while two studies using LC–ESI-MS reported linear ranges of DCAA at 1000 (90 pg–90 ng injection) and 2000 (0.1–200 µg injection) times, respectively (29,30). The difference in linearity between LC–MS and LC–MS–MS might result from a significant difference in amount injected; HAAs are highly dissociative, and ESI is usually considered as concentration dependent, so if different masses of HAAs were injected onto the inlet, there would be different concentrations of HAAs to the ESI, possibly causing dissimilar ionization efficiency. Interestingly, Bruzzoniti used IC–ESI-MS with only a 12.5-fold linearity of DCAA (50–625 ng injection) (26); Sun and Gu (36) and Yang et al. (37), analyzing HAAs with IC coupled with an electrochemical detector, reported a narrow linear range of 20 times for DCAA. The mobile phase ingredients would exert a significant influence on the linearity and ionization efficiency of ESI.

Table VII. Inter-Day Accuracy and Precision of the Method Using BetaMax Acid Column (n = 3)

HAAs	Spiked sample concentration (µg/L)	Measured concentration (µg/L) (mean ± SD)	RSD (%)*	Error (%)†
MCAA	500	594 ± 107	18.0	18.8
	2500	2529 ± 5	0.19	1.20
MBAA	25	29.0 ± 1.7	5.93	16.0
	50	56.9 ± 4.4	7.70	13.8
	500	500 ± 10	2.04	0.0
MIAA	10	11.4 ± 0.5	3.96	14.0
	25	27.2 ± 1.5	5.36	8.80
	500	520 ± 23	4.35	4.00
DCAA	3.13	3.62 ± 1.00	27.8	15.8
	10	11.8 ± 1.3	11.4	18.0
	50	50.0 ± 2.3	4.52	0.0
DBAA	5.0	6.13 ± 0.18	2.87	22.6
	25	27.0 ± 0.5	1.71	8.00
	50	48.4 ± 0.2	0.37	–3.20
TBAA	25	28.2 ± 3.3	11.8	12.8
	50	53.2 ± 2.9	5.47	6.40
	500	499 ± 5	0.91	–0.20
TCAA	5	5.97 ± 0.58	9.80	19.4
	25	24.6 ± 0.7	2.80	–1.60
	50	46.0 ± 0.2	0.52	–8.00
BCAA	1.5	1.75 ± 0.10	5.50	16.7
	10	10.6 ± 0.3	2.57	6.00
	25	25.3 ± 1.4	5.51	1.20
BDCAA	5	6.48 ± 1.36	21.0	29.6
	25	27.9 ± 3.7	13.2	11.6
	50	50.1 ± 6.3	12.6	0.20
CDBAA	25	26.8 ± 1.7	6.14	7.20
	50	52.9 ± 5.1	9.58	5.80
	100	97.3 ± 2.3	2.38	–2.70

* RSD (%) = standard deviation/mean × 100.
† Error (%) = (measured concentration – spiked concentration)/spiked concentration × 100.

Within the linear ranges, our method was accurate and reproducible. Three levels of HAAs were tested for intra- and inter-day precision and accuracy. For example, when using the BetaMax Acid column, the intra-day RSD% were within 15%, except for CDBAA at 12.5 µg/L (17.3%), and the intra-day error percents were within 12% except for TBAA at 250 µg/L (18.0%) and CDBAA at 12.5 µg/L (20.8%) (Table VI). The inter-day RSD% were within 13% except for MCAA at 500 µg/L (18.0%), DCAA at 3.1 µg/L (27.8%), and BDCAA at 5 µg/L (21.0%), and the inter-day error% were within 18% except for DBAA at 5 µg/L (22.6%), TCAA at 5 µg/L (19.4%) and BDCAA at 5 µg/L (29.6%) (Table VII).

Conclusions

The HILIC UPLC column had a better on-column LOD and made high-throughput separation possible. However, because samples had to be prepared in 90% ACN–10% water, it would be more suitable to couple with a pre-enrichment step rather than a direct injection of water samples. On the other hand, water samples could be directly injected onto the BetaMax Acid column and the major HAAs can be determined. Although the LODs of several HAAs in our methods were still not comparable with the standard method using GC–ECD, no sample preparation, including extraction, derivatization or concentration, was needed. This is the first study to provide parameters of tandem MS for all traditional nine HAAs and MIAA, and is the first to use UPLC to analyze HAAs.

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