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Short communication

Trace determination of nine haloacetic acids in drinking water by liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

A simple, fast and sensitive liquid chromatography–electrospray tandem mass spectrometry method was established for trace levels of nine haloacetic acids (HAAs) in drinking water. Water samples were removed of residual chlorine by adding L-ascorbic acid, and directly injected after filtered by 0.22 μm membrane. Nine HAAs were separated by liquid chromatography in 7.5 min, and the limits of detection were generally between 0.16 and 0.99 μg/L except for chlorodibromoacetic acid (1.44 μg/L) and tribromoacetic acid (8.87 μg/L). The mean recoveries of nine target compounds in spiked drinking water samples were 80.1–108%, and no apparent signal suppression was observed. Finally, this method was applied to determine HAAs in the tap water samples collected from five waterworks in Shandong, China. Nine HAAs except for monochloroacetic acid, monobromoacetic acid, dibromochloroacetic acid and tribromoacetic acid were detected, and the total concentrations were 7.79–36.5 μg/L. The determination results well met the first stage of the Disinfectants/Disinfection By-Products (D/DBP) Rules established by U.S.EPA and Guidelines for Drinking-water Quality of WHO.

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1. Introduction

Considerable attention had been focused on the occurrence of haloacetic acids (HAAs), a class of widespread disinfection by-products (DBPs) in drinking water, because of their suspected carcinogenicity, mutagenicity, as well as the developmental, reproductive and hepatic toxicity [1–4]. There are nine major HAAs, 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). U.S.EPA first introduced a two-stage guideline for the maximum contamination levels (MCLs) of HAAs in the Disinfectants/Disinfection By-Products (D/DBP) Rules. In the first stage, sum of MCAA, MBAA, DCAA, DBAA and TCAA (HAAs5) should not exceed 60 μg/L [5], and it will be lowered to 30 μg/L during the upcoming second stage [6]. Besides the above five HAAs, cytotoxicity and genotoxicity of TBAA have been reported to be stronger than DCAA and TCAA [7], and BCAA, DBCAA as well as BDCAA can induce the dysmorphogenesis of CD-1 mouse conceptuses, including prosencephalic and pharyngeal arch hypoplasia as well as eye and heart tube abnormalities after their exposure [3].

Gas chromatography with electron capture detection (GC-ECD) and GC equipped with mass spectrometry (GC–MS) are the commonly used methods to analyze HAAs after sample acidification, extraction and derivatization [8–12], but these two methods are tedious, labor-intensive and require a great deal of toxic organic solvent. Recently, ion chromatography (IC) is applied to HAAs analysis without derivatization [13], however it tends to suffer the interference of the ions (e.g. Cl^-, SO_4^{2-}) existing in water samples, especially after pre-concentration steps [14]. While high-field asymmetric waveform ion mobility spectrometry (FAIMS) is another relatively new technique to analyze HAAs due to its high sensitivity with little sample preparation [15,16], the instrument is not widely used in laboratories. Liquid chromatography–MS or tandem mass spectrometry (LC–MS or LC–MS/MS) is an alternative method due to its sensitivity and specificity [17]. Reversed-phase liquid chromatography (RP-LC) is the most commonly used type of LC, however, the non-polar stationary phase generally has difficulty to retain small, charged polar molecules such as HAAs [18]. Ion-pair liquid chromatography, ion-exchange liquid chromatography and hydrophilic interaction liquid chromatography (HILIC) are used to increase the retention and separation of HAAs, but pre-concentration of samples or large volume injection is necessary due to the limited instrumental sensitivity possibly caused by the suppression of additives [19–22]. Recently, Chen and Chang [23] developed an UPLC–MS/MS method based on HILIC chromatography column or C12 based column to directly analyze ten HAAs (monoiodoacetic acid was also included), unfortunately, neither of

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Table 1
Optimized instrumental and SRM conditions of HAAs.

Function (min)	Retention time (min)	Compound	Dwell time (s)	Precursor ion	Product ion	Cone voltage (V)	Collision energy (eV)
1.25–1.60	1.42	MCAA	0.07	92.6	34.8	20	11
1.50–2.25	1.58	MBAA	0.05	136.7	78.5	20	10
	1.69	DCAA	0.05	126.6	82.6	20	10
	1.81	BCAA	0.05	172.7	128.7	20	9
	1.96	DBAA	0.05	216.8	172.8	20	11
2.70–3.40	2.89	TCAA	0.05	160.6	116.6	15	8
	3.03	BDCAA	0.05	206.8	162.6	16	7
	3.15	CDBAA	0.10	250.8	206.7	15	7
3.20–3.60	3.31	TBAA	0.08	249.0	78.5	30	20

the two columns can simultaneously improve separation and signal intensity of HAAs.

In this study, we developed a sensitive and specific method for simultaneously analyzing nine HAAs in drinking water using LC–MS/MS by applying a C8 RP-LC column without the use of ion-pairing reagents and sample concentration. Finally, it was applied to the analysis of nine HAAs in the tap water samples collected from five waterworks in Shandong, China.

2. Experimental

2.1. Chemicals and reagents

Nine HAAs (purity, %), including MCAA (99.1%), DCAA (99.5%), TCAA (99.0%), MBAA (98.2%), DBAA (99.5%), TBAA (98.8%), BCAA (98.0%), BDCAA (99%), and CDBAA (99%) were all purchased from Dima Technology TNC (USA).

Methanol and acetonitrile obtained from Fisher Chemicals (New Jersey, USA) were all of HPLC grade. HPLC grade formic acid and acetic acid were purchased from Dima Technology TNC (USA). L-Ascorbic acid was analytical reagent grade and from Alfa Aesar (USA). Distilled water was prepared by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA, USA). Syringe driven filter was purchased from Anpel (China).

Stock solutions (100 mg/L in methanol) for all single standard substances were prepared, and then 10 mg/L mixture of the nine HAAs was made. The solutions in low concentrations were prepared by a series of dilutions with the Milli-Q water. All solutions were stored at 4 °C.

2.2. Sample preparation

Tap water samples from five different waterworks were all collected on January 18, 2010, in Shandong, China. All samples were added by L-ascorbic acid to 0.02 g/L for removing residual chlorine, and were stored at 4 °C. No effects on target compounds after adding L-ascorbic acid to standard solutions was observed according to the recoveries (95.3–99.3%). The result confirmed the feasibility of using L-ascorbic acid as the reagent to remove residual chlorine when analyzing HAAs. Prior to analysis by UPLC–MS/MS, water samples were filtered through 0.22 μm syringe driven filters.

2.3. Liquid chromatography and mass spectrometry

The LC apparatus was an ACQUITY UPLC™ system (Waters, Milford, MA, USA). Separation of nine HAAs was achieved using a Waters ACQUITY UPLC BEH C8 column (1.7 μm; 2.1 mm × 100 mm). The column was maintained at 40 °C and a flow rate of 0.3 mL/min, and the injection volume was 15 μL. Acetonitrile (A) and ultrapure water containing 0.1% (v/v) acetic acid (pH 3.1) (B) were used as mobile phases. The gradient was increased from initial 5% to 40% of solvent A linearly within 4 min. Then the mobile phase A was increased to 100% in another 1 min and kept for 1 min. Finally, the gradient was returned to the initial conditions of 5% A for a 1.5 min re-equilibrium before the next injection. The total run time was 7.5 min.

Mass spectrometry was performed using a Waters Micromass Quattro Premier XE (triple–quadrupole) detector equipped with an electrospray ionization source (Micromass, Manchester, UK) in the negative ion mode. The optimized MS parameters were as follows: source temperature, 110 °C; desolvation temperature, 350 °C; capillary voltage, 3.00 kV; desolvation gas flow, 800 L/h; cone gas flow, 50 L/h; and multiplier, 650 V. Finally, the data acquisition was performed in the selected reaction monitoring (SRM) mode, and time-segmented scanning in four functions was used based on the chromatographic separation of the target compounds to maximize sensitivity of detection. Flow injection analysis (FIA) was performed in order to determine the best precursor and product ion, and optimize the cone voltage and collision energy for each analyte. The precursor ion for TBAA was $[M-COOH]^-$, and $[M-H]^-$ were selected as the precursor ions for the other eight HAAs. The major product ions of MCAA, MBAA and TBAA were halogen ions $[Cl]^-$, $[Br]^-$, $[Br]^-$, respectively, while the most suitable product ions for the other six HAAs were selected as $[M-COOH]^-$ (Table 1).

2.4. Quantitation

Identification of the nine HAAs in drinking water was accomplished by comparing the retention time (within 2%) with the corresponding standards, and each sample was analyzed three times ($n = 3$). Six- to nine-point calibration curves were constructed for the standard solutions in a concentration range between 0.25 and 100 μg/L for quantification, depending on the individual compound.

To avoid sample contamination, all equipment rinses were done with methanol, and laboratory blanks were analyzed to assess potential sample contamination. Recoveries were evaluated by spiking standard solutions to a drinking water sample at three concentration levels for each HAA in replicates of three, and the original concentration was determined prior to the fortification experiment. Because no sample extraction steps were included in this method, the recovery data reflected the ion suppression. Data were analyzed using Waters MassLynx V4.1 and Microsoft Excel 2007. The limits of detection (LODs) and limits of quantitation (LOQs) were defined as signal-to-noise (S/N) ratios at 3 and 10, respectively.

3. Results and discussion

3.1. Optimizing analytical conditions

Since the LC conditions, especially the solvent conditions, can greatly influence the separation of target compounds and ESI sensitivity [24], the effects of mobile phase composition and the additives on sensitivity and separation for analyzing HAAs were investigated. In previous studies, water containing acids was the

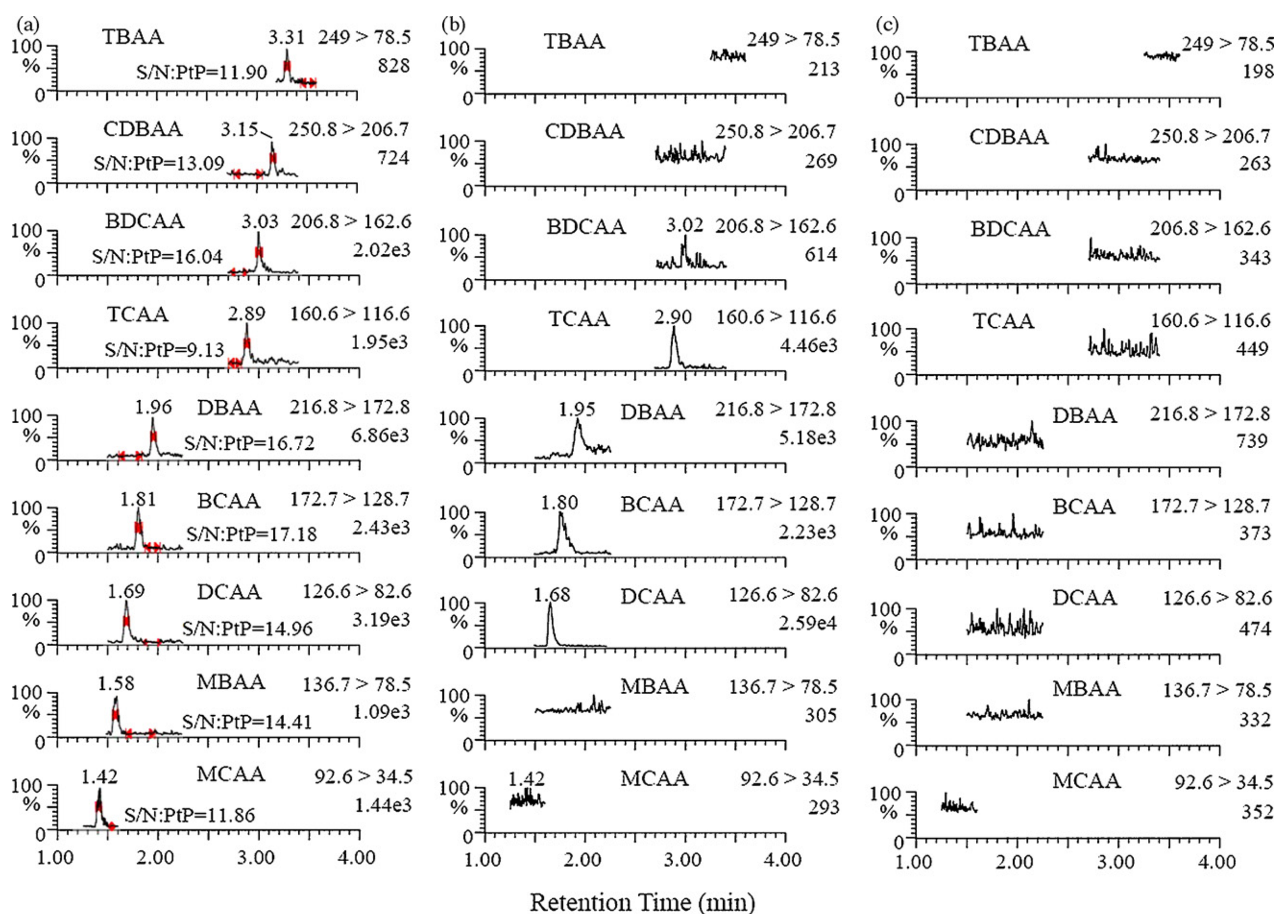

Fig. 1. LC–MS/MS SRM chromatograms of nine HAAs in (a) standard solutions (concentrations of individual HAA in standard solutions were at: DCAA, TCAA, MBAA, BCAA, 1 μg/L; MCAA, DBAA, 2 μg/L; BDCAA, CDBAA, 5 μg/L; TBAA, 25 μg/L); (b) water samples; (c) blank.

commonly used aqueous mobile phase in HAAs analysis to reduce the dissociation of HAAs and to improve their retention and separation on LC columns [21,25]. In this study, we compared acetonitrile/water containing formic acid with acetonitrile/water containing acetic acid by changing their percentage in ultrapure water at 0.05%, 0.1%, 0.2% and 0.3%. Finally, ultrapure water containing 0.1% acetic acid was used as aqueous mobile phase due to the relatively good separation and high sensitivity for most HAAs (Figs. S3–S5, Supporting Material).

Selecting a proper chromatography column is one of the most important tasks in developing a LC–MS(/MS) method, since it can greatly influence the separation and signal intensity of target compounds [19–23]. Conventional RP C18 column was reported to have difficulty to retain HAAs due to the non-polar stationary phase [18], so great efforts have been made to select a suitable column. Beta-Max Acid column containing polar embedded groups improved the retention of HAAs, however, the analytical sensitivity for certain HAAs was poor [23]. While HILIC chromatography with a Phenomenex Luna Amino column worked effectively for retention of one HAA, i.e. DCAA [26], HILIC UPLC column failed to separate nine HAAs [23]. In this study, two types of RP columns, Acquity C8 column and Acquity HSS T3 column (1.8 μm; 2.1 mm × 100 mm) were investigated to analyze HAAs, and it was found that C8 column provided better retention and separation than T3 column (Fig. S1, Supporting Material). A C18 column would theoretically provide a better retention of HAAs than a C8 column, because longer chain lengths may be more appropriate for retention of small hydrophilic molecules. Therefore, we further compared C8 column with Acquity C18 column (1.7 μm; 2.1 mm × 100 mm), and found that C8 column was actually better for capturing HAAs than C18 (Fig. S1, Supporting Material), which would be due to the fact that the longer C18 chains lay down during the early aqueous period of the gradient and therefore the hydrophilic HAAs were not captured. High retention on C8 made HAAs eluted out at higher organic portions in mobile phase, and therefore their ionizations were improved as shown in Fig. S2 (Supporting Material) where the signal intensities increased with the portion of acetonitrile in mobile phase. Thus, C8 is also better than T3 and C18 column from the view of sensitivity, and it was selected in this study.

3.2. Quantification and method validation

The method of external calibration was applied for quantification of HAAs. Calibration curves were constructed from 0.25 to 100 μg/L (the standard concentration levels for TBAA were at 10, 25, 40, 50, 80 and 100 μg/L, for MCAA and DBAA were at 0.5, 1, 2.5, 5, 10, 25, 50 and 100 μg/L, for BDCAA were at 1, 2.5, 5, 10, 25, 50 and 100 μg/L, for CDBAA were at 1.5, 2.5, 5, 10, 25, 50 and 100 μg/L, and for the other four HAAs were at 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 μg/L). Calibration graphs were linear with good correlation coefficients (R^2) all being greater than 0.99. The LODs ($n=3$) of MCAA, DCAA, TCAA, MBAA, DBAA, TBAA, BCAA, BDCAA, CDBAA were 0.35, 0.16, 0.23, 0.17, 0.32, 8.87, 0.16, 0.99 and 1.44 μg/L, respectively (RSDs were 3.0–11%), and their LOQs ($n=3$) were 2.08, 0.65, 1.09, 0.70, 1.27, 24.29, 0.56, 3.20 and 4.80 μg/L, respectively (RSDs were 0.2–10%). LOQs of all nine HAAs, especially for MCAA, MBAA, TBAA and CDBAA, were lower than those reported in the previous method without sample pre-concentration [23]. In

Table 2
Concentrations (μg/L) of HAAs in the water samples from five different waterworks.

Compound	Concentration (μg/L) [mean ± RSD (%), n = 3]				
	A	B	C	D	E
MCAA	–[a]	–	–	–	–
DCAA	5.21 ± 3.8	8.50 ± 1.8	22.5 ± 3.8	3.03 ± 2.8	5.36 ± 1.7
TCAA	3.52 ± 5.0	5.91 ± 1.9	9.71 ± 1.6	–	–
MBAA	–	–	–	–	–
DBAA	3.06 ± 2.4	1.07 ± 6.1	–	4.96 ± 1.1	–
TBAA	–	–	–	–	–
BCAA	3.65 ± 3.0	3.80 ± 3.5	2.96 ± 3.3	2.05 ± 3.2	2.43 ± 3.6
BDCAA	1.55 ± 7.0	1.64 ± 7.4	1.30 ± 4.6	–	–
CDBAA	–	–	–	–	–
HAAs9[b]	17.0	20.9	36.5	10.0	7.79
HAAs5[c]	11.8	15.5	32.2	7.99	5.36

[a] Under the detection limit.
[b] Sum of nine HAAs.
[c] Sum of MCAA, DCAA, TCAA, MBAA and DBAA.

that study, the LOQs of MCAA, MBAA, TBAA and CDBAA were 222, 11.7, 95.8 and 26.7 μg/L, respectively [23]. The above description indicated that without pre-enrichment steps, our method was sensitive enough to directly analyze major HAAs in drinking water. The intra-day and inter-day precision were calculated by the relative standard deviations (RSDs) at three concentration levels for each HAA within the linear ranges. The intra-day RSDs (n = 5) were below 8.7%. The inter-day RSDs were calculated by a 15-day period day-to-day replicated analysis, and were generally lower than 12%. The mean recoveries (n = 3) of nine HAAs in the spiked water samples were 80.1–108%, suggesting no apparent signal suppression in this study. The detailed precision and recovery data and the tested concentrations for each HAA were shown in Table S1 in Supplementary Material.

3.3. Environmental application

This method was applied to determine nine HAAs in the tap water samples collected from five waterworks in Shandong province, China. The chromatograms of HAAs in standard solutions, water samples and a blank were shown in Fig. 1, and the mean concentrations (n = 3) were listed in Table 2. Nine HAAs except for MCAA, MBAA, CDBAA and TBAA were detected in the samples. The total concentrations of nine HAAs in different water samples ranged from 7.79 to 36.5 μg/L. Of nine HAAs, DCAA, TCAA, DBAA and BCAA were the most abundant species, and the sum made up more than 90% of all HAAs concentrations. The maximum concentration of DCAA and TCAA were 22.5 and 9.71 μg/L, respectively, which were much lower than the MCLs of 50 and 100 μg/L proposed by WHO [27]. Concentrations of HAAs5 ranged from 5.36 to 32.2 μg/L, which well met the first-stage 60 μg/L [5], but that of waterworks C (32.2 μg/L) exceeded the second-stage 30 μg/L [6] standard of EPA.

4. Conclusions

A fast, sensitive and simple method was developed for directly analyzing nine HAAs in drinking water samples using UPLC–MS/MS without ion-pairing regents and sample concentration. The method provides an approach to assess the health risk of HAAs in drinking water.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.04.074.

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