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Short communication

Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization

Wenhai Chu[a], Naiyun Gao[a,*], Daqiang Yin[a], Stuart W. Krasner[b], Michael R. Templeton[c]

[a] State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Metropolitan Water District of Southern California, 700 Moreno Avenue, La Verne, CA 91750-3399, USA
[c] Department of Civil and Environmental Engineering, Imperial College London, London SW7 2AZ, UK

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ABSTRACT

The haloacetamides (HAcAms) are disinfection by-products (DBPs) in drinking water which are currently receiving increased scientific attention due to their elevated toxicity relative to regulated disinfection by-products. A simultaneous determination method of 13 HAcAms, combining solid-phase extraction (SPE) enrichment, liquid chromatographic (LC) separation, and triple quadrupole mass spectrometry (tqMS) detection with atmospheric pressure chemical ionization (APCI) using selective reaction monitoring in positive mode, was developed to measure HAcAms, including chlorinated, brominated, and iodinated analogs. Ammonium chloride and Oasis HLB were selected as the dechlorinating reagent and polymeric SPE sorbent of HAcAm samples. The used tqMS apparatus showed higher sensitivity for the studied HAcAms in the APCI mode than electrospray ionization. 13 HAcAms were separated by LC in 9.0 min, and the detection limits ranged from 7.6 to 19.7 ng/L. The SPE-LC/tqMS method was successfully applied to quantify 13 HAcAms in drinking water samples for the first time, and first indentified tribromoacetamide and chloroiodoacetamide as DBPs in drinking water.

1. Introduction

Nitrogenous DBPs (N-DBPs) formed during chlorination as part of drinking water treatment are receiving increasing attention because of the dramatically elevated toxicity of these compounds relative to many regulated DBPs without nitrogen (e.g., trihalomethanes [THMs]) [1]. DBP classes now being studied include: haloacetamides (HAcAms), halonitromethanes (HNMs), haloacetonitriles (HANs), halogenated furanones, haloaldehydes, haloquinones, as well as N-nitrosamines and iodo-DBPs [2–11]. Among these compounds, relatively few studies have focused on HAcAms.

HAcAms have been reported to be extremely cytotoxic and genotoxic in mammalian cells (142 times more cytotoxic and 12 times more genotoxic than regulated haloacetic acids [HAAs]) [2,7,8]. Plewa et al. [8] also reported that most of chlorinated and brominated HAcAms are less cytotoxic and genotoxic than their iodinated analogs. However, to-date most studies on HAcAms in drinking water have focused on the chlorinated and brominated species [2,9–13]. Five HAcAms, including chloroacetamide (CAcAm), dichloroacetamide (DCAcAm), trichloroacetamide (TCAcAm), bromoacetamide (BAcAm) and dibromoacetamide (DBAcAm) were first identified and quantified (at μg/L level) in a 2000–2002 DBP survey of 12 water treatment plants (WTPs) in the US using liquid–liquid extraction (LLE) pre-concentration and gas chromatography with electron capture detection [1,3]. In China, the formation of DCAcAm and TCAcAm was first investigated in a typical surface water treatment plant [14], using LLE separation and gas chromatography–mass spectrometry techniques. The same method detection limit of 0.1 μg/L as in the U.S. Nationwide Occurrence Study was reached [15]. Recently, some brominated HAcAms, including bromochloroacetamide (BCAcAm), bromodichloroacetamide (BDCAcAm), dibromochloroacetamide (DBCAcAm), and an iodinated HAcAm bromoiodoacetamide (BIAcAm), were identified (not quantified) from the drinking water by broad-screen analyses of GC/MS [9,10]. The occurrence of tribromoacetamide (TBAcAm) was also reported in the swimming pool water [11]. However, the concentration levels of these indentified (not quantified) and un-indentified HAcAms in real drinking water, including BCAcAm, BDCAcAm, DBCAcAm, TBAcAm, BIAcAm, iodoacetamide (IAcAm),

* Corresponding author. Tel.: +86 21 68982691.
E-mail addresses: feedwater@yahoo.cn (W. Chu), gaonaiyun@126.com (N. Gao).

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chloroiodoacetamide (ClAcAm) and diiodoacetamide (DIAcAm) are still unknown, due to the lack of the quantitative method.

HAcAms analysis presents particular challenges, as they have low volatility and mono- and di-halogenated HAcAms have significantly different polarity with their trihalogenated analogs (see Table S1 for boiling point, log K_{ow} and water solubilities of HAcAms). Liquid chromatogram/mass spectrometry (LC/MS) in conjunction with solid-phase extraction (SPE) is a well-established technique and has been used for the trace determination of numerous different classes of compounds in a variety of matrices [16–19]. In the study, we developed a novel method combining SPE enrichment and high performance LC separation with triple quadrupole MS (SPE-HPLC/tqMS) with atmospheric pressure chemical ionization (APCI) using selective reaction monitoring (SRM) in positive mode, which was used for the first time to simultaneously determine 13 HAcAms in drinking water (Fig. S1).

2. Experimental

2.1. Chemicals and materials.

CAcAm (98.5%), DCAcAm (98.5%) and TCAcAm (99%) standards were obtained from Alfa Aesar (Karlsruhe, Germany). Methanol, BAcAm and IAcAm standards were supplied by Sigma–Aldrich (St. Louis, Missouri, USA). DBAcAm, BCAcAm, BDCAcAm, DBCAcAm, TBAcAm, DIAcAm, ClAcAm, and BIAcAm standards were all purchased from Orchid Cellmark (New Westminster, BC, Canada). All other chemicals were at least analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) unless otherwise noted. The ultrapure water was produced with a Millipore Milli-Q Gradient water purification system (Billerica, USA).

2.2. SPE

Based on the known characteristics of the HAcAms (Table S1), five types of SPE cartridges were selected to extract and concentrate the 13 HAcAms from drinking water samples. The five SPE cartridges, including Oasis MCX, MAX, WCX, WAX, and HLB, were supplied from Waters (Milford, MA, USA). A GAST DOA-P504-BN oil-less vacuum pump (Michigan, USA) was used to draw the water samples through the SPE column. A typical SPE run involved conditioning and equilibrating the sorbents with 10 ml of methanol, then with 10 ml of ultrapure water at a flow rate of 5 mL/min. Once methanol was added, the SPE bed was not allowed to dry before extraction of the samples. The water samples were filtered and adjusted to pH 5 ± 0.5 to prevent the hydrolysis of HAcAms [20], and passed through the SPE cartridge at a flow rate of 3–5 mL/min (1 drop/s). After the extraction, the SPE column was washed with 5 mL of ultrapure water (5% methanol) and immediately eluted with 0.5 mL of water and 5 mL of methanol. The organic eluent was collected and concentrated down to 0.5 mL at 40 °C by a pressured nitrogen gas blowing concentrator (Youcheng Union Technology Co. Ltd., UGC-12MF, Beijing, China). The extracts were then analyzed immediately by HPLC/tqMS.

2.3. LC/tqMS

An HPLC (e2695) from Waters (Milford, MA, USA) connected to a tqMS (TSQ Quantum Access MAX) from Thermo Scientific (Waltham, MA, USA) was used to determine the 13 HAcAms. Analyst software Xcalibur was used for data acquisition and analysis. A Hypersil GOLD C18 packed column (100×2.1 mm i.d., 5 μm) with a Hypersil GOLD precolumn (10×2.1 mm i.d., 5 μm) (Thermo Scientific; Waltham, MA) was used for separation. The column temperature was controlled at 40 °C by an Alliance column heater from Waters (Milford, MA, USA). The mobile phase was composed of solvent A (ultrapure water) and solvent B (100% methanol). The solvent gradient program consisted of 5% of solvent B for 2 min, increasing solvent B from 5% to 90% over 8 min, and returning back to 5% of solvent B over 0.1 min, followed by a 5-min re-equilibration prior to the next sample injection. The flow rate was 300 μL/min. The sample injection volume was 10 μL.

After the LC separation, detection was performed by positive APCI combined with the SRM mode. The optimization of MS conditions was performed infusing a mixture of 1 mg/L HAcAms (5% MeOH:95% water) using a syringe pump. The optimal operating parameters were as follows: discharge current at 4.0 μA, vaporizer temperature at 350 °C, sheath gas pressure at 40 psi, capillary temperature at 250 °C, and collision pressure at 1.5 mTorr. Transition ions, collision energy and tube lens offset were optimized for individual analytes, as shown in Table S2.

2.4. Validation

Identification of the 13 HAcAms in water was accomplished by comparing the parent ion, fragment ion and retention time (RT) with the corresponding standards, and each sample was analyzed three times ($n = 3$). Six- to nine-point calibration curves were constructed for the HAcAm standard solutions in a concentration range between 5.0 and 200 μg/L for quantification, depending on the individual compound. Method accuracy (expressed as recovery) and precision [expressed as relative standard deviation (RSD)] were evaluated by spiking HAcAm standard solutions to the drinking water samples at three concentration levels for each HAcAm in triplicate, and the original concentration was determined prior to the fortification experiment. The limits of detection (LODs) and limits of quantification (LOQs) were defined as signal-to-noise (S/N) ratios at 3 and 10, respectively [21–23].

3. Results and discussion

3.1. Dechlorinating agents

In order to ensure that the determined results reported the HAcAm concentration at the time and point of sample collection, a dechlorinating reagent should be employed to eliminate chlorine residual and terminate the reactions between chlorine and HAcAm precursors. However, dechlorinating agents may also react with the HAcAm compounds, owing to the reducibility of common dechlorinating agents. Therefore the influence of several dechlorinating reagents that are typically used in DBP studies, including sodium sulfite, sodium thiosulfate, ascorbic acid and ammonium chloride, on the stability of 13 HAcAms was investigated. Sodium sulfite and sodium thiosulfate have been reported to dechlorinate at least two chlorinated HAcAms (DCAcAm and TCAcAm) under typical sample storage conditions, and ascorbic acid did not significantly reduce their concentrations [12]. In the present study, sodium sulfite, sodium thiosulfate and ascorbic acid all degraded HAcAm compounds to some degree, especially the brominated and iodinated HAcAms. In contrast, ammonium chloride had little influence on the stability of the 13 HAcAms over 24 h under sample storage conditions at pH = 5, probably due to the relatively low reducibility of this dechlorinating agent [24]. Ammonium chloride was therefore selected as the dechlorinating reagent of HAcAm samples.

3.2. SPE enrichment

The extraction of the HAcAms from water by SPE is difficult because of their low molecular weight and high water solubility (except for TCAcAm and TBAcAm). As shown in Table S3, the

Table 1
Recoveries and precision of 13 HAcAms from 500 mL spiked drinking water by SPE coupling with LC/tqMS.

HAcAms	0.01 μg/L		0.05 μg/L		0.5 μg/L		Precision (%)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Intra-day (n = 5)	Inter-day (n = 10)
CAcAm	71	11	85	4.7	80	3.5	1.2	3.9
DCAcAm	NE[a]	–	92	5.2	89	4.2	3.4	6.7
TCAcAm	NE	–	81	4.6	87	2.5	4.7	5.8
BAcAm	62	9.2	80	7.2	75	5.5	0.9	2.4
DBAcAm	64	6.5	77	3.4	79	4.2	2.1	7.7
BCAcAm	NE	–	78	4.3	85	3.1	5.1	7.8
BDCAcAm	NE	–	73	5.2	78	3.3	4.5	8.9
DBCAcAm	NE	–	79	4.2	75	3.5	2.4	6.4
TBAcAm	NE	–	87	3.3	83	3.2	3.7	2.1
IAcAm	65	12.4	78	5.2	72	4.4	1.5	5.3
DIAcAm	62	15.1	77	1.8	73	3.5	1.8	3.4
CIAcAm	71	–	74	4.2	75	3.8	3.5	5.5
BIAcAm	NE	–	67	6.1	62	4.3	3.4	5.7
Average	65	11	79	4.6	78	3.8	2.9	5.5

[a] Not estimated due to the low sensitivity at the fortification level tested.

highest average recoveries (78%) of the 13 HAcAms were obtained with Oasis HLB, followed by Oasis MCX (68%), MAX (50%), WCX (43%) and WAX (19%). The pH effect of water was not examined because the pH of HAcAm water samples must be kept in the 4–6 pH range to avoid the hydrolysis of HAcAms [19]. As the objective of this work was to simultaneously extract the 13 HAcAms, having different physico-chemical characteristics, SPE at pH 5 with Oasis HLB was selected as a compromise.

3.3. HPLC separation

A Hypersil GOLD C18 packed column (100 × 2.1 mm i.d., 5 μm) was chosen, which allowed an efficient chromatographic separation for all the 13 HAcAms in only 9 min. In order to further optimize the chromatographic separation, different mobile phases (methanol and acetonitrile) with different additives (HCOOH and NH_4Ac at various concentrations) were also tested. The addition of HCOOH and NH_4Ac cannot significantly improve the chromatographic separation (reduction of peak tailing and better resolution), but debased the sensitivities of some HAcAms. Compared to acetonitrile and water, the methanol and water mobile phases enhanced the chromatographic separation. Therefore, methanol and water, without the addition of HCOOH and NH_4Ac, were finally chosen as mobile phases for the simultaneous chromatographic separation of the 13 HAcAms. The ion chromatograms of the HAcAms spiked in drinking water are shown in Fig. S2.

3.4. MS optimization

Both APCI and electrospray ionization (ESI) were examined for ionization of the target HAcAms. The tqMS apparatus showed higher sensitivity for the studied 13 HAcAms when operated in the APCI mode. This is probably because APCI is much less susceptible to ion suppression, which may lead to false negative results [21]. Moreover, the APCI is usually more suitable than ESI for semi-polar compounds [25]. Therefore, APCI was used to interface the HPLC with the tqMS.

3.5. Method validation

Linearity was studied in the range 5.0–200 μg/L for all 13 HAcAms. Depending on the sensitivity reached for each HAcAm different linear responses were obtained: (1) CAcAm, BAcAm, IAcAm, DIAcAm DBCAcAm, TBAcAm, CIAcAm and BIAcAm showed satisfactory linearity along this range; (2) the rest of HAcAms showed good results from 10 to 200 μg/L. In all these cases, the correlation coefficients by linear curves were greater than 0.99.

As shown in Table S2, the LODs (n = 3) of CAcAm, DCAcAm, TCAcAm, BAcAm, DBAcAm, BCAcAm, BDCAcAm, DBCAcAm, TBAcAm, IAcAm, DIAcAm, CIAcAm, and BIAcAm were 7.16–19.7 ng/L (RSDs were 2.0–10%), and their LOQs (n = 3) were 17.2–53.6 ng/L (RSDs were 1.0–10%). When the detected concentration levels were above the LOQs, the average recoveries (n = 3) of the 13 HAcAms in the spiked drinking water samples with different concentration levels have little change (see Table 1 for 0.05 and 0.5 μg/L). The intra-day and inter-day method precision were calculated by the relative standard deviations (RSDs) at three concentration levels (10, 50, 100 μg/L) for each HAcAm within the linear ranges. The intra-day RSDs (n = 5) were below 8.9%. The inter-day RSDs were calculated by a 10-day period day-to-day replicated analysis and were generally lower than 10%, as shown in Table 1.

3.6. Analysis of real water samples

This method was applied to determine 13 HAcAms in the drinking water samples collected from seven WTPs in three provinces of China. Once collected, the HAcAm samples were quenched by ammonium chloride and adjusted to pH = 5. The analytical results are summarized in Table 2.

All 13 HAcAms, except for IAcAm and DIAcAm, were detected in the samples. The total concentrations of 13 HAcAms in different water samples ranged from 0.07 to 8.20 μg/L. Of the 13 HAcAms, DCAcAm was the most abundant species, and the sum of the three dihalogenated HAcAms (DCAcAm, BCAcAm and DBAcAm) made up more than 60% of all the HAcAms concentrations. CAcAm, DCAcAm, TCAcAm, BAcAm and DBAcAm were measured [1,2,14], and BCAcAm, BDCAcAm, DBCAcAm and BIAcAm was also indentified from drinking water previously [8,11,12]. To our knowledge this is the first ever report of the other HAcAms (TBAcAm and CIAcAm) being detected in drinking water. To identify these new HAcAms, analysis of the collected source water samples before disinfection (Table S4) was also carried out and showed no detectable HAcAms. By calculating the peak area ratios between the quantification (Q) and the two confirmation transitions (q1 and q2) (Table S2) and comparing them with ion-ratios and RT from a reference standard, we confirmed that CIAcAm and TBAcAm were formed in drinking water due to chloramination and chlorination.

Table 2
The results obtained in the monitoring of 13 HAcAms in finished water from seven urban WTPs of China.

HAcAms	Representative sample data (μg/L) [1,2]	LODs (μg/L)	Concentration (μg/L) ± RSD (%) (n = 3)						
			1	2	3	4	5	6	7
CAcAm	ND[a] ~ 0.5	0.02	ND	0.21 ± 7.8	0.29 ± 6.3	0.06 ± 8.1	ND	ND	ND
DCAcAm	ND ~ 3.9	0.04	0.07	1.51 ± 3.1	2.13 ± 3.4	1.15 ± 2.9	0.14 ± 3.5	1.57 ± 2.5	0.27 ± 2.2
TCAcAm	ND ~ 1.1	0.05	ND	0.62 ± 4.6	0.31 ± 3.9	0.07 ± 6.3	0.10 ± 4.2	0.12 ± 4.6	ND
BAcAm	ND ~ 1.1	0.02	ND	0.73 ± 3.1	1.92 ± 2.8	0.08 ± 7.2	0.12 ± 2.7	ND	ND
DBAcAm	ND ~ 2.8	0.02	ND	0.55 ± 4.7	0.76 ± 6.2	0.05 ± 5.9	ND	ND	0.21 ± 4.5
BCAcAm	Indentified/NQ[b]	0.04	ND	0.93 ± 4.3	1.34 ± 4.8	0.14 ± 4.2	0.28 ± 3.9	0.52 ± 3.5	0.14 ± 4.4
BDCAcAm	Indentified/NQ	0.06	ND	0.14 ± 6.5	0.80 ± 5.5	0.08 ± 5.3	0.12 ± 4.4	0.12 ± 5.1	ND
DBCAcAm	Indentified/NQ	0.03	ND	0.07 ± 7.6	0.22 ± 6.1	ND	ND	0.07 ± 7.6	ND
TBAcAm	Indentified[c]/NQ	0.03	ND	ND	0.15 ± 6.8	ND	ND	ND	ND
IAcAm	NI[d]	0.03	ND	ND	ND	ND	ND	ND	ND
DIAcAm	NI	0.03	ND	ND	ND	ND	ND	ND	ND
CIAcAm	NI	0.04	ND	ND	0.17 ± 7.5	ND	ND	0.08 ± 8.9	0.21 ± 7.7
BIAcAm	Indentified/NQ	0.05	ND	ND	0.09 ± 7.1	ND	ND	0.11 ± 6.2	0.15 ± 5.3

[a] ND, not detected.
[b] NQ, not quantified.
[c] Only indentified in swimming pool water.
[d] NI, not indentified.

4. Conclusions

New reliable techniques are needed for the analysis of HAcAms in drinking water. This is the first study to develop the comprehensive analysis of 13 HAcAms in drinking water, based on SPE extraction and purification followed by HPLC/tqMS analysis in APCI mode. The method provides an analytical approach to contribute to the assessment of the occurrence, formation and health risks of chlorinated, brominated and iodinated HAcAms in drinking water. This study is also the first to report TBAcAm and CIAcAm as drinking water DBPs.

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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.2012.02.074.

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