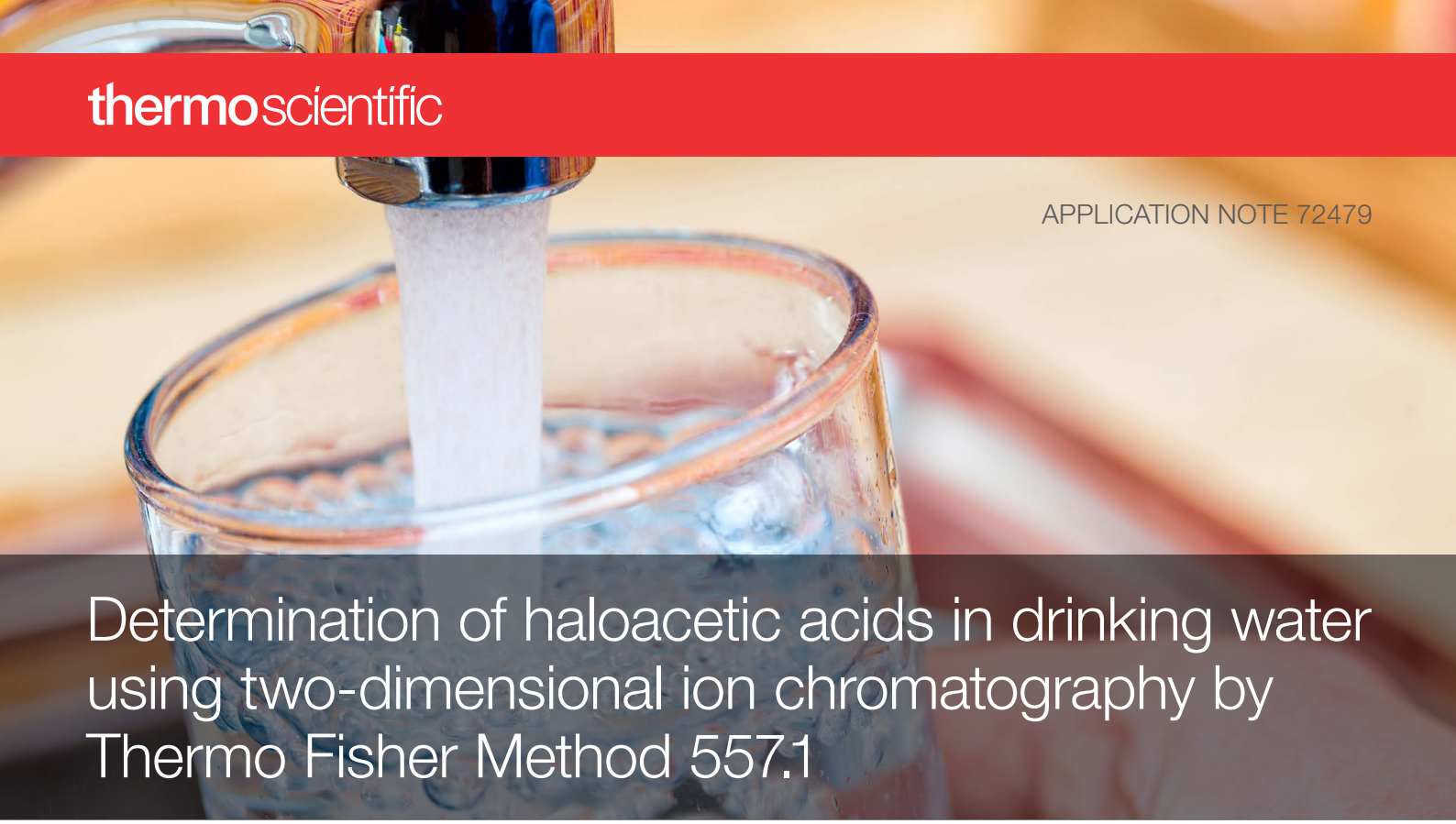

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APPLICATION NOTE 72479

Determination of haloacetic acids in drinking water using two-dimensional ion chromatography by Thermo Fisher Method 557.1

Authors

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Keywords

HAA, capillary IC, ICS-5000+, Dionex IonPac AS24A column, Dionex IonPac AS26 column, disinfection byproducts, DBPs, RFIC, 2-D IC, matrix elimination

Goal

To use two-dimensional ion chromatography to quantify haloacetic acids in deionized water, laboratory synthetic sample matrix, and drinking water

Introduction

To ensure the safety of drinking water, it is routinely treated with ozone or other chemical disinfectants, such as chlorine or chloramine, to kill pathogenic microorganisms (e.g., *E. coli* and *Giardia lamblia*). An undesirable consequence of this treatment is the formation of disinfection byproducts (DBPs), which arise from the reaction of disinfectants with bromide and inorganic or organic matter (such as decaying vegetation). These DBPs include bromate, chlorite, chlorate, trihalomethanes (THMs), and haloacetic acids (HAAs; Table 1). Because they can have a detrimental impact on human health, for example, by causing cancer, damaging the nervous system, and introducing birth defects[1], the World Health Organization[2] has established guidelines for DBPs in drinking water. In the U.S., these guidelines are regulated by the Environmental Protection Agency (EPA) as part of the Safe Drinking Water Act (SDWA). In 1998, the Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR) was published, which set the limit for total trihalomethanes (TTHM) at 80 µg/L and, for the first time, set the maximum contamination levels for five of the HAAs (HAA5) at 60 µg/L. It also set a maximum contaminant level goal (MCLG) for dichloroacetic acid (DCAA) to zero and trichloroacetic acid (TCAA) to 0.3 mg/L. In the Stage 2 DBPR, the MCLG for TCAA was reduced to 0.02 mg/L and monochloroacetic acid (MCAA) was set at 0.07 mg/L.[3]

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Table 1. Haloacetic acids (HAA9). HAA6Br, HAAs containing bromine; HAA5, HAAs currently regulated by the U.S. EPA.

		Acid	HAA	Formula
HAA5	HAA9	Monochloroacetic Acid	MCAA	$ClCH_2CO_2H$
		Dichloroacetic Acid	DCAA	Cl_2CHCO_2H
		Trichloroacetic Acid	TCAA	Cl_3CCO_2H
		Monobromoacetic Acid	MBAA	$BrCH_2CO_2H$
		Dibromoacetic Acid	DBAA	Br_2CHCO_2H
		Tribromoacetic Acid	TBAA	Br_3CCO_2H
	HAA6Br	Bromochloroacetic Acid	BCAA	$BrClCHCO_2H$
		Chlorodibromoacetic Acid	CDBAA	Br_2ClCCO_2H
		Bromodichloroacetic Acid	BDCAA	Cl_2BrCCO_2H

As part of the SDWA, the Unregulated Contaminant Monitoring Rule (UCMR) was established in which the U.S. EPA is required every five years to create a list of not more than 30 contaminants to be monitored in public water systems serving > 10,000 people.[4] During each monitoring cycle, a Candidate Contaminant List (CCL) is evaluated for inclusion in the upcoming UCMR, pre-implementation activities occur, data are collected, and then the results analyzed to determine if any of the contaminants monitored should be considered for establishment of regulatory limits. For UCMR4, which will run from 2017–2021, three groups of HAAs will be monitored: HAA5, HAA9, and HAA6Br (Table 1).

The U.S. EPA methods that have been approved for the determination of HAAs in drinking water are 552.1[5], 552.2[6], 552.3[7], and 557[8]. The 552 methods require pH adjustment, solvent extraction, methylation, another extraction, and then measurement by gas chromatography using electron capture detection. EPA Method 557 avoids the extraction and methylation steps by using direct injection of sample onto the anion exchange column of an ion chromatography (IC) system and monitoring by suppressed conductivity. Unwanted matrix components are diverted to waste while the HAAs are directed to a tandem mass spectrometer for quantification.[9]

An alternative method that does not require multiple extraction steps or the use of a mass spectrometer is described in Thermo Fisher Method 557.1[10] and uses direct injection of water samples onto a two-dimensional IC (2-D IC) system to quantify HAAs. In the first dimension, a 4 mm i.d. column is used to separate sample components so that matrix can be diverted to waste and analytes focused onto a concentrator column. A smaller i.d. (0.4 mm) second dimension column (resulting in a higher mass response) is then used to resolve and quantify HAAs. There are two other EPA methods that use 2-D IC, one for bromate[11] and another for perchlorate[12] (302.0 and 314.2, respectively).[13,14]

In this application note, 2-D IC was used to quantify HAAs in reagent water (RW), laboratory synthetic sample matrix (LSSM), and drinking water from surface water (SW) and ground water (GW) sources. A large sample volume (500 µL) was injected onto and separated on a 4 mm i.d. high-capacity Thermo Scientific™ Dionex™ IonPac™ AS24A column followed by matrix diversion to waste and focusing of suppressed effluent containing HAAs onto a Thermo Scientific™ Dionex™ IonSwift™ MAC-200 Monolith Anion concentrator column. Analytes were then resolved and quantified on a 0.4 mm i.d. Thermo Scientific™ Dionex™ IonPac™ AS26 column. Both column sets used had selectivity optimized for 15 °C separation, a temperature that minimizes temperature-induced analyte degradation. The second dimension had selectivity different from the first, providing greater confidence in peak identification. Additionally, the multiple extractions required by GC methods are avoided, while the detection levels are comparable to those of the IC-MS/MS method, without the complications that can be associated with MS detection.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ Hybrid (Analytical/Capillary) HPIC™ system, including:
  - DP Dual Pump
  - EG Eluent Generator
  - DC Detector/Chromatography module (low temperature, P/N 22171-60008) with Thermo Scientific™ Dionex™ ICS-5000+ IC Cube™ containing a six-port valve (P/N 078841) and CD Conductivity Detector
  - Thermo Scientific™ Dionex™ AS-AP Autosampler with a 5.0 mL sample syringe (P/N 074308) and 8.5 mL buffer line assembly (P/N 075520) and diverter valve (P/N 074123)
- Thermo Scientific™ Dionex™ AXP Auxiliary Pump (P/N 063973)
- Thermo Scientific™ Dionex™ Peristaltic Pump (P/N 064508)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2

Consumables

Analytical

- Thermo Scientific™ Dionex™ EGC 500 KOH Potassium Hydroxide Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 Continuously Regenerated Anion Trap Column (P/N 075550)
- Thermo Scientific™ Dionex™ CRD 300 Carbonate Removal Device, 4 mm (P/N 064637)
- Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Suppressor, 4 mm (P/N 082540)

Capillary

- Dionex EGC KOH (Capillary) Eluent Generator Cartridge (P/N 072076)
- Dionex CR-ATC Continuously Regenerated Anion Trap Column (Capillary) (P/N 072078)
- Dionex CRD 200 Carbonate Removal Device, 0.4 mm Capillary (P/N 072054)
- Thermo Scientific™ Dionex™ ACES™ 300 Anion Capillary Electrolytic Suppressor (P/N 072052)

Storage Vials

- Amber Collection Vials, 60 mL (P/N 048781)
- Amber VOA Glass Vials, 20 mL (Fisher Scientific P/N 139-20A)
- Amber Vials, 2 mL, 500 pk (Fisher Scientific P/N 6000.0060; storage of standard stock solutions transferred from shipping ampules)
- Screw caps, 2 mL, 500 pk (Fisher Scientific P/N 6000.0057)
- Septa (silicone) 2 mL, 500 pk (Fisher Scientific P/N 6000.0058)

Autosampler vials

- Vial kit, 10 mL, Polystyrene with caps and blue septa (P/N 074228)

Reagents and standards

- Reagent (Deionized (DI)) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Fisher Scientific, ACS Grade Glacial Acetic Acid (P/N A38)
- Fisher Scientific, ACS Grade Ammonium Chloride (P/N A661)
- Fisher Scientific, Certified Grade Sodium Hydroxide, 50% w/w solution (P/N SS254)
- Restek® Corporation (First Source Standard) Haloacetic Acid Mix, 1,000 µg/mL each in methyl tert-butyl ether (P/N 31896)
- Supelco® (Second Source Standard) EPA 552.2 Haloacetic Acids Mix, 2,000 µg/mL each in methyl tert-butyl ether (P/N 49107-U)

Samples

Ground and surface waters were collected from locations in Ohio and Kentucky into bottles containing ammonium chloride to give a final concentration of 100 mg/L. Samples were stored at 4 °C.

Conditions	
First dimension	
Columns:	Dionex IonPac AG24A Guard, 4 × 50 mm (P/N 076011) Dionex IonPac AS24A Analytical, 4 × 250 mm (P/N 076010)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
Gradient:	KOH: 7 mM (0–12 min), 7 to 14 mM (12–32 min), 65 mM (32.1–50 min; HAA5) or (32.1–60 min; HAA9)
Flow Rate:	1.0 mL/min
Temperature:	13 °C (Upper compartment)
	15 °C (Lower compartment)
Autosampler Tray Temp.:	5 °C
Inj. Volume:	500 µL
Detection:	Suppressed conductivity, Dionex AERS 500 Anion Electrolytically Regenerated Suppressor, external water mode, 161 mA
Background Conductance:	< 0.3 µS
Noise:	< 0.3 nS/min peak-to-peak
System Backpressure:	~2300 psi
Run Time:	50 min (HAA5) or 60 min (HAA9)

Conditions	
Second dimension	
Columns:	Dionex IonPac AG26 Capillary Guard, 0.4 × 50 mm (P/N 076019) Dionex IonPac AS26 Capillary Analytical, 0.4 × 250 mm (P/N 076018)
Eluent Source:	Dionex EGC-KOH (Capillary) Eluent Generator Cartridge with Dionex CR-ATC Continuously Regenerated Anion Trap Column (Capillary)
Gradient:	HAA5: 5.2 mM KOH (0–53 min), 155 mM (53.1–65 min) HAA9: 5.2 mM KOH (0–53 min), 155 mM (53.1–60 min), 100 mM (60.1–80 min)
Flow Rate:	0.012 mL/min
Temperature:	13 °C (Upper compartment) 15 °C (Dionex IC Cube Cartridge)
Cut Volume:	~23 mL (HAA9), 14 mL (HAA5)
Concentrator:	Dionex IonSwift MAC-200 Monolith Anion Concentrator Column, 0.75 × 80 mm (P/N 075461)
Detection:	Suppressed conductivity, Dionex ACES 300 Anion Capillary Electrolytic Suppressor, external water mode, 25 mA
Background Conductance:	< 1 µS
Noise:	< 1 nS/min peak to peak
System Backpressure:	~1600 psi
Run Time:	65 min (HAA5), 80 min (HAA9)

Preparation of solutions and reagents

Haloacetic acids primary dilution standard (PDS)

Prepare a 1.0 µg/mL HAA solution by adding 0.050 mL of the 1,000 µg /mL stock standard to a 50 mL volumetric flask and dilute to volume with DI water.

Note: Because of the volatility of the analyte stock solution (HAAs in methyl tert-butyl ether) it is best to chill (to ~4ºC) the DI water used for dilution (ensuring no contamination from the chilling process) immediately prior to use.

Haloacetic acids secondary dilution standard (SDS)

Prepare a 100 µg/L haloacetic acids solution by adding 1.0 mL of PDS to a 10 mL volumetric flask and diluting to volume with DI water.

Haloacetic acids calibration standards

Prepare haloacetic acids calibration standards by adding the appropriate volumes of the primary or secondary dilution standards to 50 mL volumetric flasks and then 100 mg/L ammonium chloride to the 50 mL mark (Table 2).

Use a calibration range of 0.05–2 µg/L for Lowest Concentration Minimum Reporting Level (LCMRL) calculations. A higher range of 0.5–20 µg/L is appropriate for a method reporting limit of 0.5 µg/L in drinking waters. To minimize the introduction of contaminants to the standard solutions, rinse the 60 mL amber collection vials three times with DI water and allow to drain prior to transferring solutions. Following the mixing of standards, add a few milliliters of the mixture to the DI-water-rinsed amber vials, swirl, and then discard to waste. This will reduce the likelihood that any residual DI water in the vial will dilute the standard. Add remainder of standard dilution to the vial.

Second source quality control sample (QCS)

To verify the accuracy of the primary calibration standards, a solution containing the method analytes at 5 µg/L is prepared from a source that is different from the source used for the calibration standards. Prepare the second source 1 µg/mL PDS by adding an appropriate amount of standards stock (0.025 mL of 2,000 µg /mL Supelco standard was used here) to a 50 mL volumetric flask and dilute to volume with DI water. To prepare the QCS, dilute 250 µL of the second source PDS into 50 mL final volume of 100 mg/L ammonium chloride in a volumetric flask.

Acid wash solution

Prepare a 100 mM acetic acid solution by adding 1.5 g of acetic acid to a 250 mL volumetric flask and diluting to volume with DI water.

Base wash solution

Prepare a 100 mM sodium hydroxide solution by adding 2.0 g of 50% sodium hydroxide to a 250 mL volumetric flask and diluting to volume with DI water.

Laboratory synthetic sample matrix (LSSM)

LSSM is a high ionic strength solution that sets the upper limit for salt content in water samples that can be analyzed by this method. Prepare the LSSM so that the final concentrations are: nitrate (20 mg/L), bicarbonate (150 mg/L), chloride (250 mg/L), and sulfate (250 mg/L), based on the masses of the anions (Table 3).

Table 2. HAA standard mixtures.

Dilution Standard	Dilution Standard Vol. (µL)	Final Vol. (mL, 100 mg/L Ammonium Chloride)	Final Concentration (µg/L)
	1000	50	20
	500	50	10
	250	50	5.0
PDS	100	50	2.0
	50	50	1.0
	40	50	0.80
	25	50	0.50
	125	50	0.25
SDS	50	50	0.10
	25	50	0.05

Table 3. Preparation of concentrated LSSM stock (10 ×).

Compound	Salt (gfw)[a]	Anion (gfw)	Salt Mass (mg)	Volume DI Water (L)	Stock Conc. (mg/L)[b]	LSSM (mg/L)
Nitrate	84.99	62.00	137	0.5	200	20
Bicarbonate	84.01	61.02	1,030	0.5	1,500	150
Chloride	58.44	35.45	2,060	0.5	2,500	250
Sulfate	142.04	96.06	1,850	0.5	2,500	250

[a]gfw = gram formula weight of the sodium salt. [b]10 x concentrated stock.

The ammonium chloride preservative is also included in the LSSM matrix. To a 1 L graduated container, add 100 mL of LSSM stock and 100 mg ammonium chloride. Fill with DI water to mark and mix thoroughly.

Store all solutions and reagents at 4 °C.

Sample preparation

At the time of collection, 100 mg/L ammonium chloride is added to all water samples to prevent chlorine-mediated formation of method analytes and microbial degradation during storage. Consequently, all standards are prepared in ammonium chloride preservation solution rather than DI water. Prepare the ammonium chloride preservation solution by dissolving 100 mg of ammonium chloride in 1 L of DI water for a final concentration of 100 mg/L. Store at 4 °C.

To minimize contamination, soak the 10 mL polypropylene autosampler vials and blue septa in DI water and then allow to dry prior to use.

System preparation and configuration

For the analytical system, install, and configure the EG by first installing a yellow PEEK backpressure restrictor tubing (P/N 053765) in place of the columns to produce a total backpressure of 2000–2500 psi at a flow rate of 1 mL/min. Install a Dionex EGC 500 KOH Eluent Generator cartridge and condition the cartridge by setting the KOH concentration to 50 mM at 1 mL/min for 30 min. After the conditioning is complete, disconnect the backpressure tubing that was temporarily installed in place of the column set. Install a Dionex CR-ATC Continuously Regenerated Anion Trap Column between the Dionex EGC 500 KOH cartridge and the Dionex EGC degasser. Hydrate the Dionex CR-ATC trap column prior to use by following the instructions outlined in the Dionex Eluent Generator Cartridges (EGC) QuickStart Guide.[15]

For the capillary system, connect a 38 cm (15 in.) piece of 0.025 mm (0.001 in.) i.d. PEEK tubing between the pump pulse damper and the Dionex EGC Eluent Generator Cartridge inlet port to provide the backpressure for the eluent generator cartridge configuration procedure. Set the pump flow rate to 0.02 mL/min and flush the capillary Dionex EGC cartridge for 20 min. After flushing is complete, connect the capillary Dionex CR-ATC Continuously Regenerated Anion Trap Column at the outlet of the Dionex EGC Eluent Generator Cartridge and flush the Dionex CR-ATC trap column at 0.02 mL/min for 15 min. To condition the Dionex EGC cartridge and Dionex CR-ATC trap column, set the KOH concentration to 50 mM at 0.02 mL/min for 15 min. Detailed instructions for plumbing the capillary system can be found in the Dionex ICS-5000+ Ion Chromatography System Installation Instructions Manual.[16]

Install and configure the Dionex AS-AP Autosampler in Sequential Push Mode. Follow the instructions in the Dionex AS-AP Autosampler Operator's Manual[17] to calibrate the sample transfer lines to ensure accurate and precise sample injections. Due to the large sample injection volumes in this application, install a 5 mL sample syringe. An 8.5 mL buffer line assembly is also required. Prepare a 500 µL sample loop by measuring ~43.2 in. of 0.030 in. i.d. tubing. To verify the volume of the loop, weigh the empty tubing, fill the tube with DI water, then reweigh the filled tube and calculate the volume. The total sample volume must be 500 µL ± 5%. Install a diverter valve on the Dionex AS-AP Autosampler. Connect Port 1 of the diverter valve to Port 5 on the System #1 injection valve (first dimension; Figure 1). Connect the diverter valve port 2 (for concentrator rinses) to Port 3 of the Auxiliary valve (second valve in the DC lower compartment). The Auxiliary valve is used to control cuts (i.e. the column effluent from the first dimension to the concentrator column).

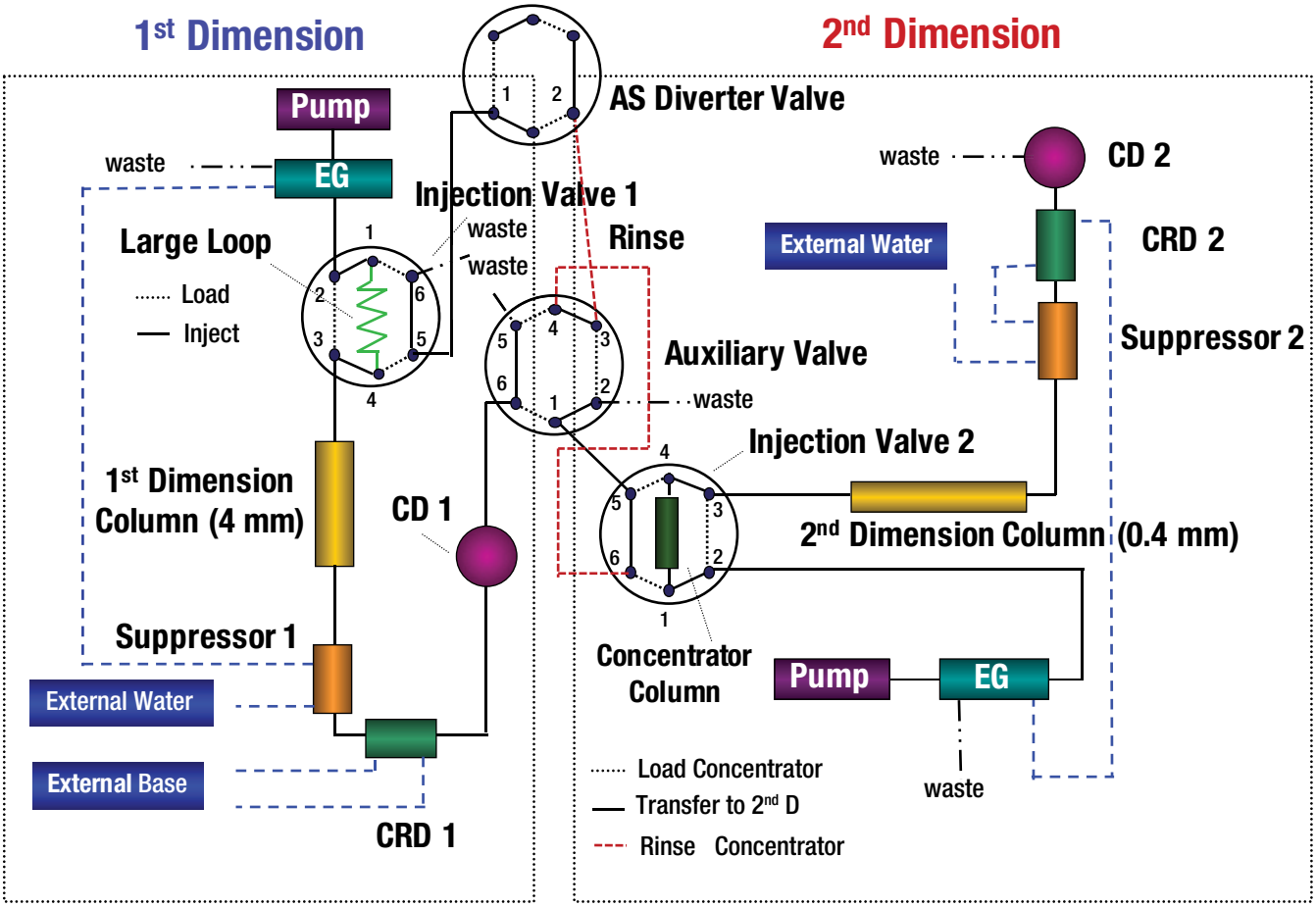

Figure 1. Schematic diagram of the 2-D IC system showing flow paths.

Install the sample loop on Injection Valve #1 of the DC. A six-port valve is required on the Dionex IC Cube cartridge to replace the four-port valve for System #2.

Install the Dionex IonPac AG24A and the Dionex IonPac AS24A columns on System #1 in the lower compartment of the DC. For System #2, install the Dionex IonPac AG26 and the Dionex IonPac AS26 capillary columns in the column cartridge and install the cartridge in the Dionex IC Cube module in the upper compartment of the DC. When installing new columns, after connecting the column inlet, direct the outlet to waste and pump eluent through it at a standard flow rate for the column for ~30 min before connecting to the suppressor.

Connect a piece of 0.01 in. i.d. tubing from the conductivity detector cell out on System #1 to Port 6 on the Auxiliary valve. Port 1 on the auxiliary valve is connected to Port 5 on the injection valve of the Dionex IC Cube cartridge. The length of this tubing should be kept to a minimum. Port 4 on the auxiliary valve is connected to Port 6 on the injection valve of the Dionex IC Cube cartridge using a piece of 0.02 in i.d. tubing.

Install a Dionex IonSwift MAC-200 concentrator column in place of the sample loop on the injection valve of the Dionex IC Cube cartridge so that the direction of sample loading is opposite to the direction of the capillary flow. i.e. Connect so that the flow direction arrow on the column points from Port 1 to Port 4.

Hydrate the Dionex AERS 500 and Dionex ACES 300 suppressors according to the instructions in the QuickStart and operating manual.[18,19] Operate both suppressors in the external water mode by connecting the external water source to the Regen In of the suppressor, the Regen Out of the suppressor to the Regen In of the Dionex CR-ATC trap column, and the Regen Out of the Dionex CR-ATC trap column to the Regen In of the EG degasser. For the analytical

suppressor, set the peristaltic pump to deliver a flow rate of ~2 mL/min. This same pump is also used to recirculate the NaOH used as regenerant for the carbonate removal device (see below). For capillary, use the Dionex AXP Auxiliary Pump to flow the external water at 0.1 mL/min. A plumbing diagram showing the Dionex ICS-5000⁺ HPIC system setup is depicted in Figure 2.

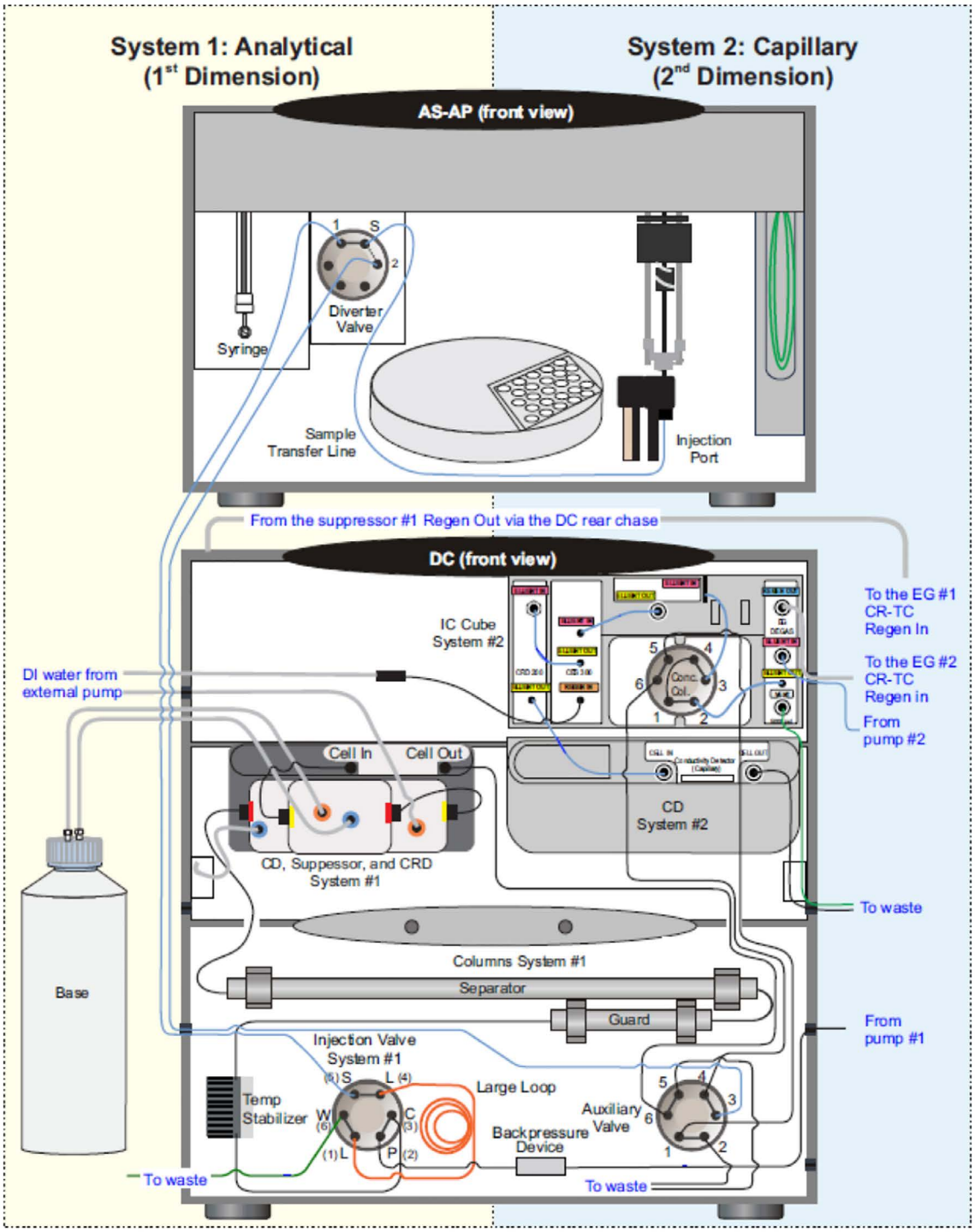

Figure 2. Plumbing diagram of the 2-D IC setup using the Dionex ICS-5000⁺ HPIC system.

Hydrate the Dionex CRD 300 4mm and CRD 200 capillary Carbonate Removal Devices according to the instructions in the operating manual[20] and QuickStart.[21] Operate the CRD 300 4mm Carbonate Removal Device in the external base mode by connecting the external base (2 L of 200 mM NaOH) to the Regen In and the Regen Out back to the external base, using the same peristaltic pump as the analytical suppressor external water flow to deliver ~2 mL/min.

Equilibrate the Dionex IonPac AS24A column set with 55 mM KOH and the Dionex IonPac Capillary AS26 column set with 65 mM KOH at their respective flow rates (shown in the Conditions section) for ~60 min. Analyze a water blank by injecting DI water. An equilibrated system has a background conductance of ~0.3 µS and ~0.8 µS for the analytical and capillary systems, respectively.

Concentrator column acid and base washes

One instrument method controls the first dimension and a second method controls the second dimension. Before each run, a third method (wash) is used for separate base and acid washes of the concentrator that must be completed prior to sample loading. The wash solutions are delivered by the Dionex AS-AP autosampler through the diverter valve (position 2) and consist of 1 mL of 100 mM sodium hydroxide, followed by 1 mL of 100 mM acetic acid.

Determining the cut time for the second dimension

Due to small variations in system plumbing, column capacity, and tubing lengths, individual laboratories must establish the optimum cut times from the first dimension before determining HAAs in the second dimension. This is accomplished by injecting 1 mg/L HAA calibration standard in 100 mg/L ammonium chloride with (A) and without (B) LSSM onto the first dimension. The first cut window width should be set to encompass peaks 1 and 2 (mono-halogenated), the second, peaks 3-5 (di-halogenated), and the third, peaks 6-9 (tri-halogenated). The cut windows should begin 0.5 min before the start of the first peak (in LSSM) and end 0.2 min after the last peak (no LSSM). For HAA5, the last cut window should end 0.2 min after the end of the TCAA peak. The 1 mg/L standard should be run weekly on the first dimension to verify the accuracy of the cut windows and times adjusted as needed.

Results and discussion

For HAA determinations, the ability of the Dionex ICS-5000+ HPIC system to precisely and consistently maintain the columns at 15 °C is critical. This temperature needs to be maintained because, at the high pH used in anion exchange chromatography, HAAs may degrade,[8] which would result in an underestimation of the concentration of these analytes. Additionally, the HAAs MBAA, CDBAA, and TBAA degrade at ambient temperature, which requires that samples be held at < 10 °C until they can be injected for analysis (i.e., a temperature-controlled autosampler is required). One further consideration that 2-D IC avoids is the need to optimize the ion transfer tube and vaporizer temperatures of an MS when using IC-MS/MS because high MS source temperature causes a loss in TCAA signal.[9]

Cut window determination

One mg/L of HAA standard mixture in LSSM is used to establish the start of each cut window and the same concentration in the absence of LSSM is used to determine the end (Figure 3). Based on the HAA peak positions, the first cut window was from 13.8–19.7 min, the second from 27.0–35.9 min, and the third from 40.8–55.5 min (for HAA9). For HAA5, the last cut window ended at 44 min.

Columns:	Dionex IonPac AG24A/AS24A, 4 mm i.d.
Flow Rate:	1.0 mL/min
Eluent:	7 mM KOH (0–12 min), 7 to 14 mM (12–32 min), 65 mM (32.1–60 min)
Eluent Source:	Dionex EGC 500 KOH cartridge
Detection:	Suppressed conductivity, Dionex AERS 500 Suppressor, 4 mm, 161 mA
Inj. Volume:	500 µL
Temp.:	15 °C
Sample:	1 mg/L HAA9 in A. LSSM B.100 mg/L NH_4Cl

Peaks:

14–20 min	1. MCAA	6. TCAA	*41–43 min (H AA5)*
	2. MBAA	7. BDCAA	
	3. DCAA	8. CDBAA	*41–56 min (HAA9)*
27–36 min	4. BCAA	9. TBAA	
	5. DBAA		

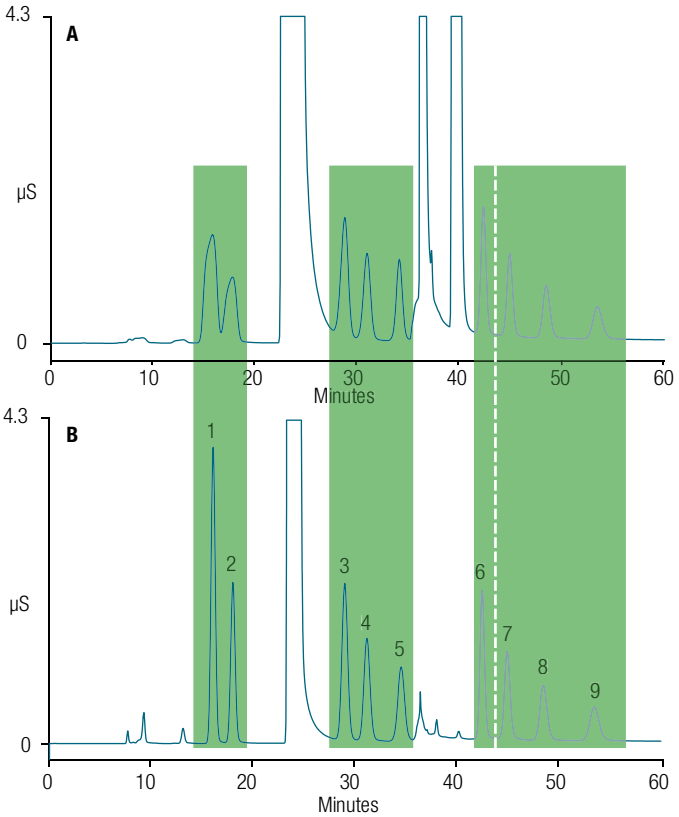

Figure 3. First dimension cut windows used to define transfer of HAA5 and HAA9 to the concentrator column. The dashed vertical line represents the end of the cut window if only HAA5 are to be determined.

Following transfer of the HAAs from the first dimension onto the concentrator column, the analytes were resolved on the second dimension and the peak areas determined (Figure 4).

Columns:	Dionex IonPac AG26/AS26, 0.4 mm i.d.
Flow Rate:	0.012 mL/min
Eluent:	5.2 mM KOH (0–53 min) 155 mM (53.1–60 min) 100 mM (60.1–80 min)
Eluent Source:	Dionex EGC KOH capillary cartridge
Detection:	Suppressed conductivity, Dionex AERS Anion Capillary Electrolytic Suppressor, 25 mA
Concentrator:	Dionex IonSwift MAC-200 column
Temp.:	15 °C
Sample:	20 µg/L HAA9 in 100 mg/L NH_4Cl

Peaks:

1. MCAA	6. TCAA
2. MBAA	7. BDCAA
3. DCAA	8. CDBAA
4. BCAA	9. TBAA
5. DBAA	

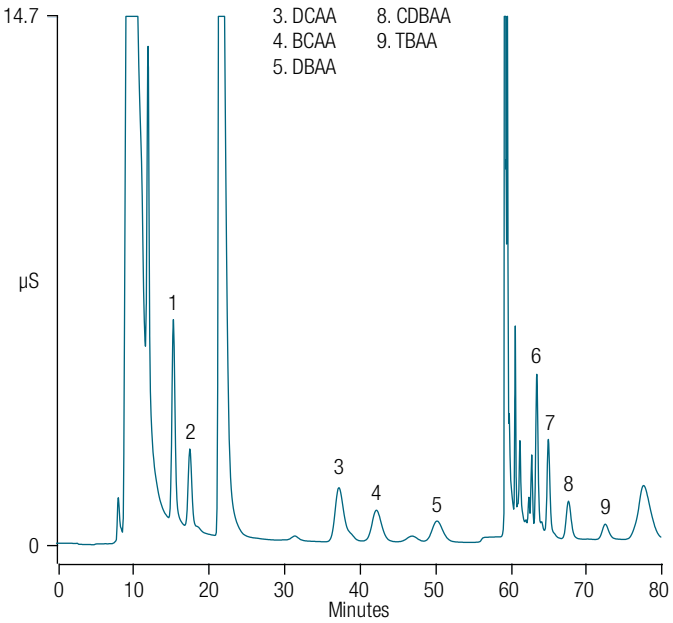

Figure 4. Second dimension separation of HAA9 following concentration of 20 µg/L HAA standards injected onto the first dimension.

Calibration curves

The calibration plots of peak area versus concentration were fitted using a quadratic relationship for concentrations that ranged from 0.05 to 2 µg/L. The coefficients of determination (r^2) were all at or above 0.999, ranging from 0.9986 (BDCAA) to 1.000 (DBAA; Table 4). The fit is exemplified by the MCAA curve, which had an r^2 of 0.9995 (Figure 5). Using these calibrations, it was determined that the concentration of HAAs in the second source QCS (5 µg/L HAAs) ranged from 4.6 to 5.1 µg/L for HAA5 with RSDs from 0.33 to 1.5 (n=3; data not shown) verifying the validity of the calibration curves from the first HAA standard source. For the LCMRL calculations, a calibration range of 0.05–2 µg/L was used, but for determination of HAAs in drinking water, 0.5–20 µg/L was used. For this concentration range, coefficients of determination were at or above 0.999.

Table 4. Coefficients of determination for 0.05–2 µg/L HAAs (quadratic fit).

HAA	r^2
MCAA	0.9995
DCAA	0.9999
TCAA	0.9998
MBAA	0.9997
DBAA	1.000
TBAA	0.9994
BCAA	0.9999
CDBAA	0.9998
BDCAA	0.9986

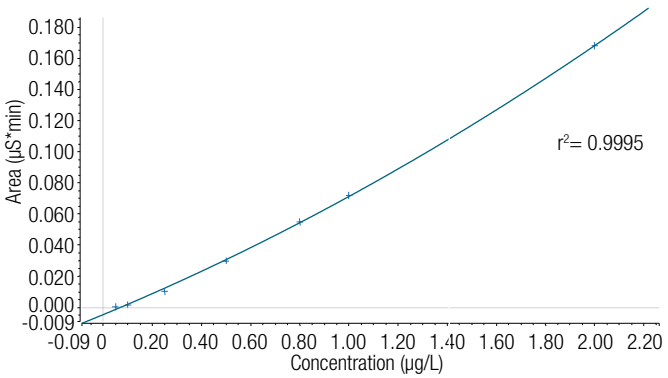

Figure 5. MCAA calibration curve. A quadratic curve fit was used.

LCMRL determinations

The Lowest Concentration Minimum Reporting Level (LCMRL) is the lowest spiking concentration at which recovery of 50–150% is expected 99% of the time. LCMRLs were calculated by entering the concentrations for each of the HAAs obtained from four replicate injections of HAA standard (0.05–2 µg/L) into the U.S. EPA's LCMRL calculator.[22] The calculated LCMRLs ranged from 0.055–0.41 µg/L, which was comparable to those values included in EPA method 557 (Table 5).

Table 5. HAA LCMRLs determined using the EPA LCMRL calculator[22] and those published in EPA Method 557.[8]

HAA	LCMRL (µg/L)	
	Calculated	EPA Method 557
MCAA	0.085	0.58
DCAA	0.41	0.13
TCAA	0.26	0.25
MBAA	0.10	0.19
DBAA	0.090	0.062
TBAA	0.28	0.27
BCAA	0.30	0.16
CDBAA	0.055	0.080
BDCAA	0.29	0.19

Continuing calibration check standards (CCCs)

To verify the accuracy of the existing calibration, standard solutions containing the method analytes at the low-, mid-, and high-level (0.5, 5, and 20 µg/L, respectively) of the calibration curve are analyzed at the start, during, and at the end of each analysis batch. The values obtained for the CCCs during all the batches were within the range of ± 0.25, 1.25, and 6 µg/L for each level, respectively.

HAAs in drinking water from ground and surface water sources

Reagent water had HAA concentrations that were below the lowest calibration level used (0.05 µg/L). This was expected because of the extensive purification used to produce the reagent water. In contrast, drinking water from a ground water source contained HAAs that ranged in concentration from 0.4 (MCAA) to 1.8 (BCAA) µg/L (Figure 6(A) and Table 6).

First Dimension

Columns:	Dionex IonPac AG24A/AS24A, 4 mm i.d.
Flow Rate:	1.0 mL/min
Eluent:	7 mM KOH (0–12 min) 7–14 mM (12–32 min) 65 mM (32.1–60 min)
Eluent Source:	Dionex EGC 500 KOH cartridge
Detection:	Suppressed conductivity, Dionex AERS 500 suppressor, 4 mm, 161 mA
Inj. Volume:	0.5 mL
Temp.:	15 °C
Sample:	A: Ground water B: Sample A + 10 µg/L HAA9

Second Dimension

Columns:	Dionex IonPac AG26/AS26, 0.4 mm i.d.
Flow Rate:	0.012 mL/min
Eluent:	5.2 mM KOH (0–53 min) 155 mM (53.1–60 min) 100 mM (60.1–80 min)
Eluent Source:	Dionex EGC KOH capillary cartridge
Detection:	Suppressed conductivity, Dionex ACES suppressor, 25 mA
Concentrator:	Dionex IonSwift MAC-200 column
Temp.:	15 °C

Peaks:		
	1. MCAA	6. TCAA
	2. MBAA	7. BDCAA
	3. DCAA	8. CDBAA
	4. BCAA	9. TBAA
	5. DBAA	

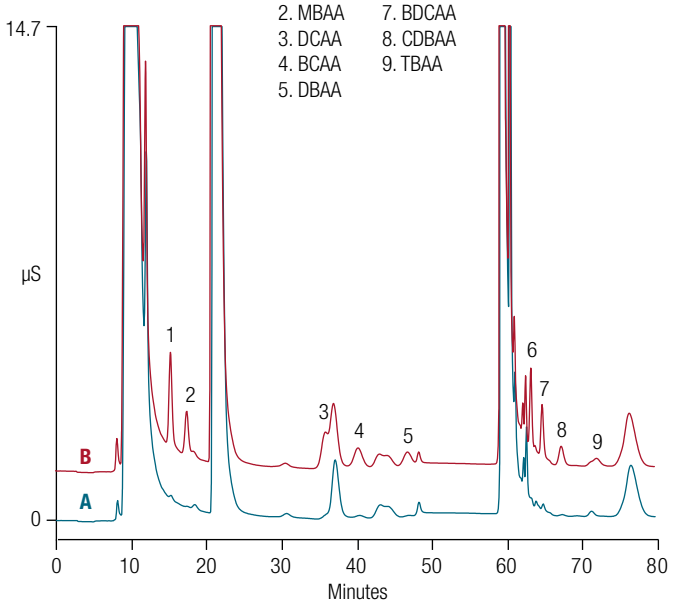

Figure 6. Determination of HAAs in drinking water derived from ground water.

First Dimension

Columns:	Dionex IonPac AG24A/AS24A, 4 mm i.d.
Flow Rate:	1.0 mL/min
Eluent:	7 mM KOH (0–12 min) 7–14 mM (12–32 min) 65 mM (32.1–60 min))
Eluent Source:	Dionex EGC 500 KOH cartridge
Detection:	Suppressed conductivity, Dionex AERS 500 suppressor, 4 mm, 161 mA
Inj. Volume:	0.5 mL
Temp.:	15 °C
Sample:	A: Surface water B: Sample A + 10 µg/L HAA9

Second Dimension

Columns:	Dionex IonPac AG26/AS26, 0.4 mm i.d.
Flow Rate:	0.012 mL/min
Eluent:	5.2 mM KOH (0–53 min) 155 mM (53.1–60 min) 100 mM (60.1–80 min)
Eluent Source:	Dionex EGC KOH capillary cartridge
Detection:	Suppressed conductivity, Dionex ACES suppressor, 25 mA
Concentrator:	Dionex IonSwift MAC-200 column
Temp.:	15 °C

Peaks:		
	1. MCAA	6. TCAA
	2. MBAA	7. BDCAA
	3. DCAA	8. CDBAA
	4. BCAA	9. TBAA
	5. DBAA	

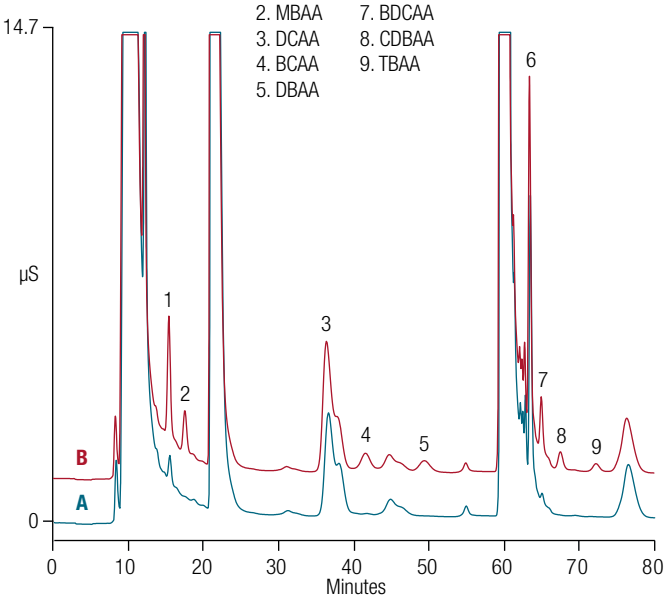

Figure 7. Determination of HAAs in drinking water derived from surface water.

In DW from a SW source, the concentrations of MBAA, TBAA, and BDCAA were comparable to that in DW from GW, but three HAAs were considerably higher (MCAA (6 x), DCAA (30 x), and TCAA (40 x); Figure 7(A), Table 6). Some HAAs were also lower (DBAA (0.3), BCAA (0.1), and CDBAA (0.1). This elevation in HAAs was likely due to the exposure of surface water to the environment compared to the relative isolation of water from a ground water source. SW has much greater direct exposure to decaying vegetation and sunlight, which can influence the formation of HAAs.

Precision and accuracy

The precision of the method was determined by running seven replicates of HAAs spiked into water at 2.5 and 10 µg/L. Because of the elevated levels of DCAA and TCAA (>30 µg/L) in SW samples, these were diluted five-fold prior to fortification so that the concentrations determined were below the highest calibration standard (20 µg/L). The relative standard deviation (RSD) for the amount ranged from 0.29–8.0% for the 2.5 µg/L HAA spike (Table 7) and was generally lower for the 10 µg/L HAA spike, ranging from 0.20 to 2.98% (Table 8).

Table 6. HAAs in drinking water from ground and surface water sources.

HAA	Ground Water		Surface Water	
	Average Concentration (µg/L)	RSD	Average Concentration (µg/L)	RSD
MCAA	0.409	0.97	2.46	0.47
DCAA	1.04	7.72	31.6	0.57
TCAA	0.806	1.88	34.4	0.17
MBAA	0.410	1.92	0.382	0.33
DBAA	0.762	3.30	0.238	1.00
TBAA	0.647	2.23	0.527	1.48
BCAA	1.82	1.82	0.256	8.31
CDBAA	0.978	0.47	0.0943	14.3
BDCAA	1.54	2.18	1.87	0.22

n=3

Table 7. Recovery of 2.5 µg/L HAAs in reagent water, LSSM, and drinking water from ground water (GW) and surface water (SW) sources (n = 7). *SW samples were diluted five-fold prior to fortification.

HAA	Reagent Water		LSSM		Drinking (Ground)		Drinking (Surface)*	
	Recovery (%)	RSD	Recovery (%)	RSD	Recovery (%)	RSD	Recovery (%)	RSD
MCAA	91.6	1.50	90.6	0.35	91.9	3.41	84.4	0.99
DCAA	87.0	3.40	88.3	3.04	77.8	1.88	96.9	0.76
TCAA	88.3	0.37	88.3	0.37	99.1	1.54	96.1	0.60
MBAA	103.3	0.35	110.3	0.53	110.6	0.52	96.0	1.25
DBAA	90.7	3.77	90.0	8.03	100.8	1.54	106.2	1.88
TBAA	96.5	1.61	106.8	0.89	87.6	3.33	98.9	1.83
BCAA	94.3	4.37	93.0	4.07	90.3	1.99	100.7	1.54
CDBAA	88.9	0.29	89.7	0.63	97.3	0.57	85.2	1.39
BDCAA	102.5	3.29	102.1	1.03	101.7	0.79	89.7	1.17

Table 8. Recovery of 10 µg/L HAAs in reagent water, LSSM, drinking water from ground (GW) and surface water (SW) sources (n = 7). *SW samples were diluted five-fold prior to fortification

HAA	Reagent Water		LSSM		Drinking (Ground)		Drinking (Surface)*	
	Recovery (%)	RSD	Recovery (%)	RSD	Recovery (%)	RSD	Recovery (%)	RSD
MCAA	96.3	0.61	95.1	0.41	92.9	1.53	92.6	1.63
DCAA	99.3	0.26	99.3	0.48	87.3	0.32	94.2	0.85
TCAA	100.8	0.63	100.1	0.97	103.5	0.28	96.1	0.75
MBAA	106.1	1.88	112.1	2.20	115.9	0.36	103.3	1.51
DBAA	97.2	1.54	96.2	2.98	101.4	0.88	103.0	1.45
TBAA	98.4	0.20	98.3	0.51	108.4	0.72	95.6	1.63
BCAA	98.3	0.33	98.5	0.42	101.7	0.96	101.6	1.55
CDBAA	99.2	0.29	99.1	0.50	102.7	0.20	95.3	1.67
BDCAA	105.3	0.27	104.0	0.82	103.5	0.18	98.3	1.58

The accuracy of the method was evaluated by determining the recoveries from the HAA spikes. Recoveries were all well within the generally accepted range of ±30% for EPA methods such as 557, ranging from 77.8% to 110% with an average of 95% for 2.5 µg/L and from 87.3% to 115.9% with an average of 100% for 10 µg/L.

Conclusion

HAAs were determined with sensitivity, precision, and accuracy in reagent water, lab synthetic sample matrix, and drinking water from ground and surface water sources by following Thermo Fisher Method 557.1 using 2-D IC with a Dionex ICS-5000⁺ HPIC system. Direct sample injection avoided the need for multiple extractions and methylation, a requirement for EPA Method 552, which can add considerable time to the analysis and requires the use and disposal of toxic solvents. There is also not the additional expense associated with using a mass spectrometry system.

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