APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Rapid Analysis of Haloacetic Acids in Drinking Water Using UHPLC/MS/MS

Introduction

Haloacetic acids (HAAs) are a class of undesired by-products resulting from the reaction of chlorinated disinfectants with natural organic matter in water. The resulting substances are considered major disinfection by-products (DBP), which are known for being carcinogenic and also associated with reproductive problems in humans. The five major HAAs of particular concern include monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), dibromoacetic acid (DBAA) and trichloroacetic acid (TCAA). Haloacetic acids are regulated by the U.S. Environmental Protection Agency (EPA) government and the maximum contaminant level (MCL) set by The United States EPA is 30 µg/L. Traditionally, gas chromatographic (GC) technology was mainly used to determine HAAs in water, which requires laborious sample preparation steps such as liquid-liquid extraction and derivatization, prior to GC analysis with mass spectrometry (MS) for detection. In 2012, the Japan Ministry of Health revised the inspection method for HAAs, which led to expansion of the analytical technique to include liquid chromatography combined with tandem mass spectrometry (LC/MS/MS) as an alternative to the GC-MS method. The LC/MS/MS method offers a simpler approach, which does not require derivatization of any of the haloacetic acids.

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In this application note, we present a simple, reliable and fast method for determination and quantification of five HAAs in potable drinking water using a PerkinElmer QSight® LX50 UHPLC system coupled to a PerkinElmer QSight 220 MS/MS detector. The five HAAs eluted within three minutes with excellent reproducibility (< 5 %RSD). The linear dynamic ranges for all compounds were within 0.1 to 20 µg/L with excellent regression coefficient ($R^2 \geq 0.999$). The established limits of quantification (LOQ) were less than 0.1 µg/L. The current UHPLC/MS/MS method permits for direct sample injection with negligible sample preparation, thus, greatly improves the laboratory productivity for HAAs analysis in water.

Experimental

Hardware and Software

Chromatographic separation and subsequent detection were carried out using the PerkinElmer QSight LX50 ultra high performance liquid chromatograph and the QSight 220 series tandem mass spectrometer respectively. All instrument control, analysis and data processing were performed using the Simplicity™ 3Q software platform.

Solvents, Standards, and Sample Preparation

LC/MS grade methanol (MeOH) and water used for the analysis were obtained from Thermo Fisher Scientific®. Each haloacetic acid standard was obtained from Toronto Research Chemicals (Toronto, ON, Canada). All other reagents were purchased from Sigma-Aldrich® (Oakville, ON, Canada).

Stock solutions were prepared separately by accurately weighing 5 mg of each HAA standard and dissolved in 5 mL of MeOH. The stock solutions were kept in the refrigerator until usage. All the stock standards were initially combined to make an intermediate stock solution, which was subsequently used for the preparation of calibration standards. Calibration standard solutions (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 µg/L) were prepared daily in amber glass vials and sealed with polytetrafluoroethylene (PTFE) cap.

Except for filtering, all potable drinking water samples were submitted for UHPLC/MSMS analysis without any further sample preconcentration/preparation step. Recovery experiments were carried out at two concentration levels (1.0 and 10 µg/L) by spiking to blank water sample. Replicate experiments were carried out for the recovery analysis.

Method Parameters

The LC method and MS parameters are presented in Table 1 and 2, respectively. Table 2 is further divided into 2a (MRM transitions with their respective optimized voltages) and 2b (MS source parameters). The MRM transitions, collision energies (CE), entrance voltages (EV) and collision cell lens 2 (CCL2) for each analyte were detected and optimized by direct infusion of the standards. MS source conditions such as drying and nebulizer gas flow and temperature settings were optimized by flow injection analysis (FIA) method.

Table 1. LC Method Parameters.

Column	**PerkinElmer Universal C18, 150×4.6 mm, 5 µm (PN: N9304757)**					
Mobile Phase	Solvent A: Water Solvent B: Acetonitrile					
	Step	**Time (min)**	**Flow Rate (mL/min)**	**%A**	**%B**	**Curve**
	1	Initial	0.6	70	30	
	2	0.25	0.6	70	30	Linear
	3	2.00	0.6	10	90	Linear
	4	2.20	0.6	10	90	Linear
	5	2.30	0.6	5	95	Linear
	6	3.00	0.6	2	98	Linear
	7	3.20	0.6	2	98	Linear
	8	3.30	0.6	70	30	Linear
	9	7.00	0.6	70	30	
Analysis Time	3.3 min; Re-equilibration time: 3.7 min					
Oven Temp.	40 °C					
Injection Volume	10 µL					

Table 2. MS Method Parameters.
(a). MRM Transitions

Compound		Precursor Ion (m/z)	Product Ion (m/z)	EV(V)	CCL2 (V)	CE (eV)
Monochloroacetic acid	MCAA	93.1	35.1	12	15	18
Monobromoacetic acid	MBAA	137.1	79.1	10	34	12
Dichloroacetic acid	DCAA	127.1	83.1	5	32	18
Dibromoacetic acid	DBAA	217.1	172.9	10	52	13
Trichloroacetic acid	TCAA	161.1	117.1	10	32	13

(b) MS Source Parameters

Parameter	Setting Value
Ionization Mode	ESI negative
Drying Gas Setting	120
HSID Temperature (°C)	100
Nebulizer Gas Setting	350
Electrospray Voltage (V)	-3800
Source Temperature (°C)	300

Results and Discussion

For improved separation and sensitivity, several analytical columns and mobile phase conditions (i.e. with or without ammonium acetate or formate additives) were evaluated for the target compounds. All analytical columns tested during optimization showed very minimal differences in retention times profiles for the analytes. Finally, a PerkinElmer Universal C18 (150×4.6 mm, 5 µm) was selected for best performance in terms of analyte peak shape, retention and signal intensity. The optimized mobile phases used for the analysis were methanol and water. All the five haloacetic acids eluted under three minutes (results shown in Table 3).

Table 3. Chromatographic retention times for haloacetic acids.

Compound	Ret. Time (min)	
Monochloroacetic acid	MCAA	1.87
Monobromoacetic acid	MBAA	1.94
Dichloroacetic acid	DCAA	1.83
Dibromoacetic acid	DBAA	1.90
Trichloroacetic acid	TCAA	2.10

Figure 1 shows a typical overlay of chromatograms for MCAA, MBAA, DCAA, DBAA and TCAA.

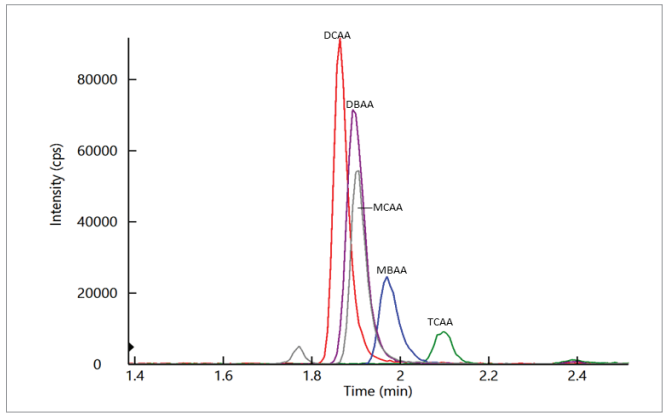

Figure 1. Typical chromatogram of MCAA, MBAA, DCAA, DBAA and TCAA of 5 µg/L.

Linearity and Limit of Quantitation

Linearity obtained for each analyte was over two orders of magnitude from 0.1 to 20 µg/L, with regression coefficient (r^2) ≥ 0.999 in the potable drinking water matrix.

Figure 2 shows examples of calibration curves for all five HAAs. Limit of quantitation (LOQ) were calculated based on a signal-to-noise ratio ≥ 10 for the quantifier MRM transitions of all analytes. In all cases, the LOQs for the 5 HAAs were ≤ 0.1 µg/L, which were more than 50 times lower than the regulated amounts.

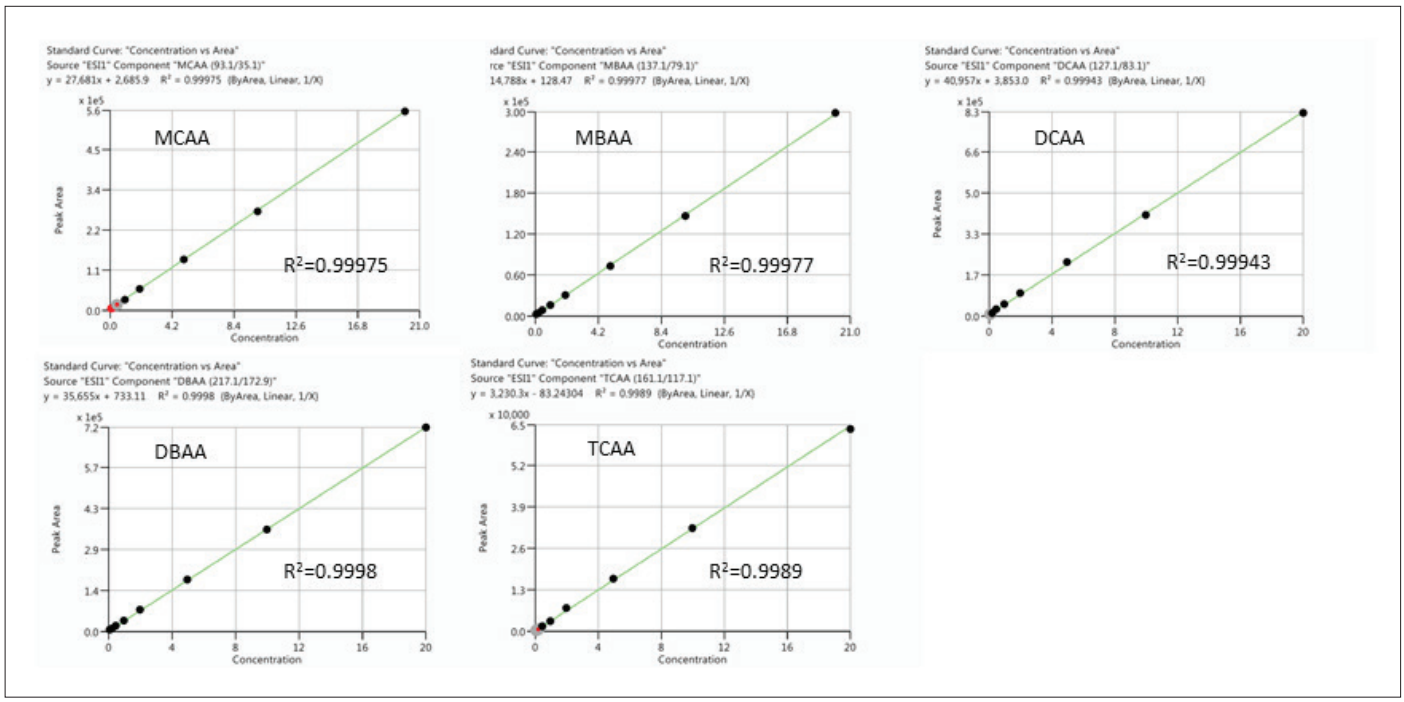

Figure 2. Calibration curves for MCAA, MBAA, DCAA, DBAA and TCAA.

HAA Recovery

Percent recovery for each analyte was determined by spiking known amounts of HAAs in blank water sample after initial filtration. The analytes percent recoveries were between 82 % to 100 %, with a % RSD < 5 for eight replicate injections. As a proof of concept, percent recoveries for analytes in the 10 µg/L spiked sample were higher than 1.0 µg/L sample. In addition, relative recoveries among chloroacetic acids decreased with increasing molecular weight. A similar trend was observed for bromoacetic acids.

Table 4. Average recoveries and % RSD at 1 and 10 µg/L.

Compound	% Recovery 1.0 µg/L Spike (n=8)	% RSD 1.0 µg/L Spike (n=8)	% Recovery 10 µg/L Spike (n=8)	% RSD 10 µg/L Spike (n=8)
MCAA	99.2	2.6	99.9	1.2
MBAA	100.3	2.8	100.1	1.2
DCAA	85.7	3.2	99.9	0.7
DBAA	84.7	3.2	96.8	1.3
TCAA	82.4	4.6	97.0	2.5

Conclusion

An easy, fast and robust LC/MS/MS method for HAAs analysis in potable drinking water sample was developed by coupling a UHPLC system to a triple quadrupole mass spectrometer. The developed method was applied to analysis of five haloacetic acid residues in potable drinking water samples. The recorded LOQs were well below the limits set by regulatory boards.

No prior sample preparation was required except sample filtration process using PTFE filter. The percent recovery for all analytes were within 82–100% with reproducibilities (% RSD) < 5 % for all HAAs even at 1µg/L level of concentration.

The overall results clearly demonstrate the applicability and effectiveness of the method for analyzing haloacetic acid residues in portable drinking water samples.

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