

Quantification of Haloacetic Acids in Tap Water Using a Dedicated HAA LC Column with LC-MS/MS Detection

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Key Words

Haloacetic acid, Water Supply Act, LC-MS/MS, TSQ Quantum Ultra, Acclaim HAA HPLC column

Goal

To develop an LC-MS/MS method for measuring haloacetic acids in tap water using a dedicated HPLC column.

Introduction

In April 2012, methods provided by the Japanese Ministry of Health, Welfare and Labour based on provisions in the Water Quality Standards Ordinance (Ministry of Health, Welfare and Labour, Notification 261, July 2003) were revised, and the inspection method for haloacetic acids (HAAs) was expanded to include an analysis method using liquid chromatography paired with mass spectrometry (LC/MS or LC-MS/MS) as an alternative to gas chromatography with mass spectrometry (GC/MS).

The LC-MS(/MS) method does not require derivatization of samples and is therefore a simple measurement method. However, tap water typically contains on the order of several to several dozen mg/L of chloride, sulfate, carbonate, and nitrate anions. When performing LC-MS analysis, these anions inhibit the ionization of haloacetic acids and cause signal suppression in the MS detector. In addition, when using a standard reversed-phase column, the retention varies depending on matrix differences, the infusion amount, and the column lot, resulting in poor recoveries, robustness, and detection limits.

A number of LC-MS/MS methods for haloacetic acids using C18 (ODS) columns have already been developed. However, separation from the many ionic matrix components contained in tap water has been insufficient in these methods. Retention times varied widely between neat standards and real samples, making it difficult to obtain reproducible results.



The Thermo Scientific™ Acclaim™ HAA column is designed for analyzing haloacetic acids in drinking water by LC/MS. It is based on mixed-mode column technology and offers reversed-phase and anion-exchange retention mechanisms that enable separation of haloacetic acids in high ion matrices. This results in robust performance in real drinking water samples that contain matrix ions. In addition, sample preparation costs are reduced because analysis is possible without sample preparation or concentration.

This application note describes the LC-MS(/MS) separation using the Acclaim HAA column for haloacetic acid analysis in drinking water.

Experimental

Sample Preparation

Preparation of standards

A methyl tertiary-butyl ether (MTBE) solution combining four types of haloacetic acids [monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), and monobromoacetic acid (MBAA), 100 mg/L of each, Kanto Kagaku] was used for the haloacetic acid standard solution. The solution was diluted in ultrapure water and used to prepare the calibration curve.

Preparation of laboratory fortified matrix

The following anions were added to a final concentration as shown: Cl^- , 35 mg/L; SO_4^{2-} , 35 mg/L; NO_3^- , 50 mg/L. Ascorbic acid was added at 10 mg/L level.

Preparation of the sample

After sampling the tap water, ascorbic acid was added at the level of 10 mg/L for residual chlorine removal.

Liquid Chromatography

Equipment:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system, which included the LPG-3400RS Quaternary Rapid Separation Pump, WPS-3000TRS Rapid Separation Thermostatted Wellplate Sampler, and TCC-3000RS Rapid Separation Thermostatted Column Compartment
Column:	Acclaim HAA column (2.1 x 50 mm, 3 μm), P/N SP6917
Mobile phase A:	Water (LC/MS grade)
Mobile phase B:	200 mM aqueous ammonium sulfate solution
Mobile phase C:	Acetonitrile
Gradient:	Refer to Figure 1
Flow rate:	0.3 mL/min
Operating temperature:	25.0 °C
Injection volume:	50 μL

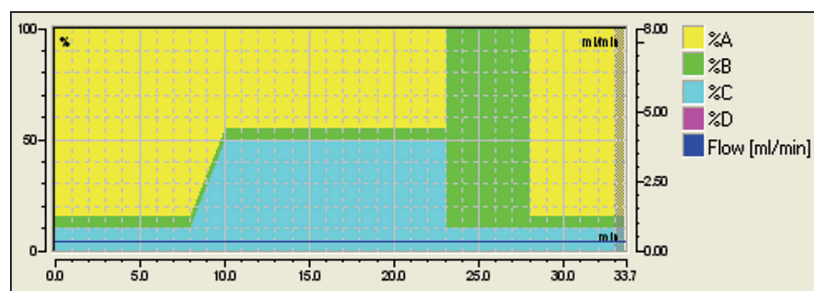


Figure 1. LC gradient

To extend the life of the columns, they should be stored in a 100 mM acetic ammonium (pH 5.0)/acetonitrile (1:4 v/v) solution.

Mass Spectrometry

Equipment:	Thermo Scientific™ TSQ Quantum Ultra™ triple-stage quadrupole MS
Ionization method:	Negative ESI
Spray voltage:	500 V
Sheath gas:	60 arbitrary units
Aux gas:	10 arbitrary units
Capillary temperature:	250 °C
Vaporizer temperature:	400 °C
Skimmer offset:	10 V
Collision gas pressure:	Ar, 0.8 mTorr
Cycle time:	2 ms
Mass resolution:	Q1: 1.5 Da (SRM mode)
SRM transitions:	Refer to Table 1

Table 1. SRM transitions

Compound Name	Precursor (m/z)	Product (m/z)	CE (eV)
MCAA quantitation ion	93	35	10
MCAA qualifying ion	95	37	10
DCAA quantitation ion	127	83	10
DCAA qualifying ion	129	85	10
TCAA quantitation ion	161	117	10
TCAA qualifying ion	163	119	10

Results and Discussion

Separation of Matrix Ions

When optimizing separation conditions, it is important to adequately separate matrix ions and haloacetic acids. However, further care is required to separate chloride ions and MCAA. Detection close to the MCAA retention time was confirmed using accurate mass MS.¹ Cl^- (m/z 35) is detected as fragment ions from NaCl_2^- (m/z 93) using CID. It is therefore detected in the same transition as MCAA. If the retention mechanism is unclear, or if separation of MCAA and chloride cannot be confirmed, false quantification could result, depending on the behavior of the chloride ions. This is why processing to remove chloride ions is recommended in analysis systems using ODS columns.

In this investigation for the Acclaim HAA column, the resolution of HAAs from interfering anions in a synthetic sample matrix spiked with HAAs is demonstrated (Figure 2).

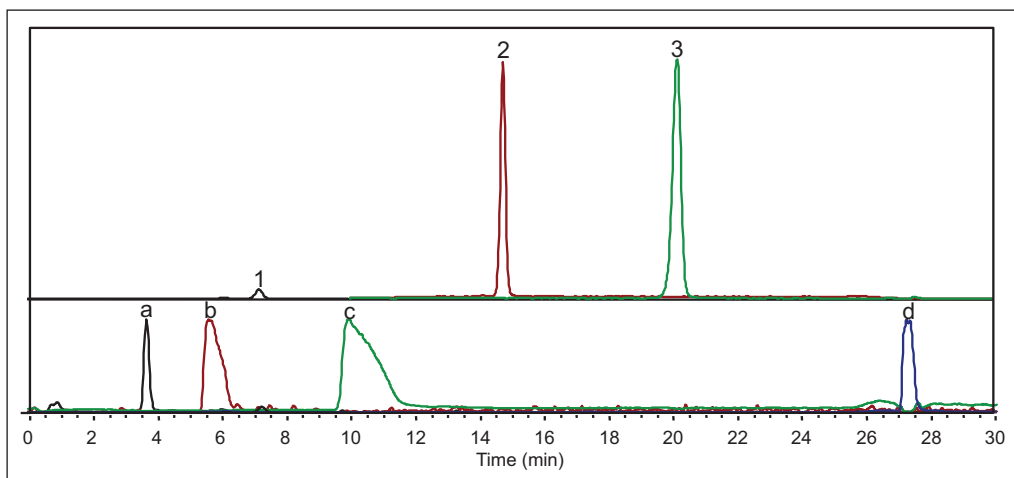


Figure 2. Separation on the Acclaim HAA column. Upper section: Haloacetic acids 1: Monochloroacetic acid (MCAA), 2: Dichloroacetic acid (DCAA), 3: Trichloroacetic acid (TCAA). Lower section: Matrix components a: Ascorbic acid, b: Chloride, c: Nitrate, d: Sulfate

Sensitivity

Based on the Japanese Water Quality Standards Ordinance, the method needs to be able to detect three regulated haloacetic acids at concentration levels ten times lower than the regulated amounts. Among the three haloacetic acids, the compound MCAA has lowest regulated amount at 20 µg/L. The HAA column method was able to confirm all three haloacetic acids at the 2 µg/L level, which is ten times lower than 20 µg/L (Figures 3 and 4).

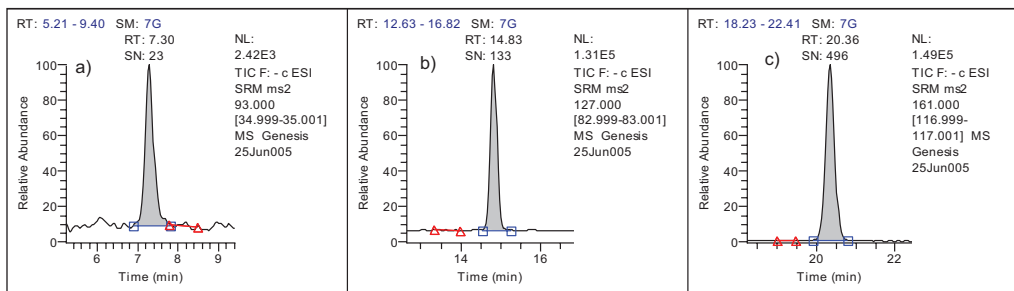


Figure 3. SRM chromatograms for 2 µg/L standard solution: a) MCAA, b) DCAA, c) TCAA

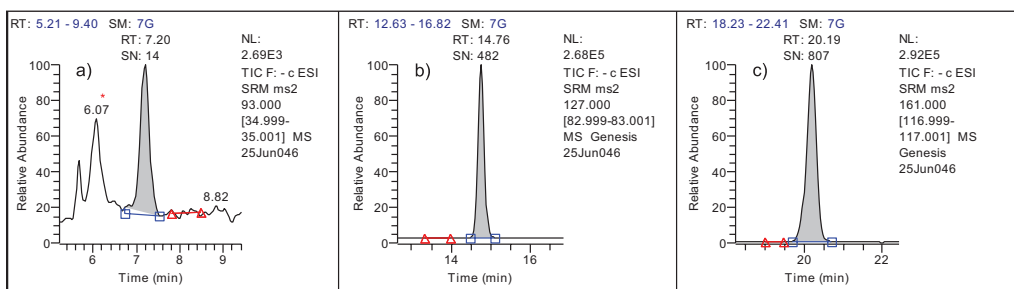


Figure 4. SRM chromatograms for tap water spiked with 2 µg/L standard solution: a) MCAA, b) DCAA, c) TCAA

*A peak from contaminant compound sources was detected before the peak in monochloroacetic acid. The identity of this contaminant as a chloride ion cluster ion (NaCl_2^-) was confirmed using a Thermo Scientific accurate-mass MS.

Calibration Curve and Reproducibility

The calibration curves were created over a range from 1 to 20 µg/L with linearities greater than 0.99 (Figure 5). Reproducible results were obtained for 2 µg/L of standard solution and for tap water spiked with 2 µg/L of standard solution. Coefficients of variation were less than 1.6% (Table 2).

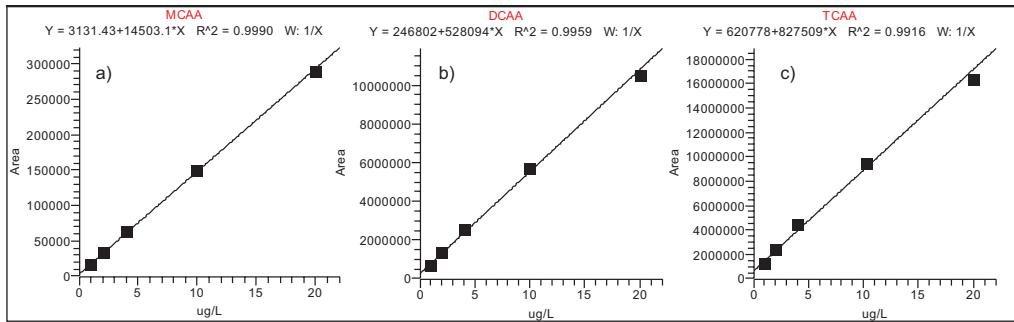


Figure 5. Calibration curves: a) MCAA, b) DCAA, c) TCAA

Table 2. Area value reproducibility for 2 µg/L of standard product and 2 µg/L of spiked tap water

	MCAA		DCAA		TCAA	
	Standard 2 µg/L	2 µg/L of spiked tap water	Standard 2 µg/L	2 µg/L of spiked tap water	Standard 2 µg/L	2 µg/L of spiked tap water
Blanks (ultrapure or tap water)	NF	6136	NF	2013784	NF	3602064
n=5 (area value)	33492	33192	1369465	3000890	2434743	5301570
	32335	33581	1355191	3079005	2465008	5241249
	32605	34016	1361893	3083660	2476721	5325868
	33295	34005	1381170	3059149	2472097	5257085
	33406	34025	1387243	3061388	2474253	5281431
%CV	1.6%	1.1%	1.0%	1.1%	0.7%	0.6%

Confirmation of Recovery Level

A spike recovery test was performed for the tap water sample spiked with standards to 2 µg/L. Good recoveries in the range of 92% to 101% were obtained (Table 2). The same test was performed for the spiked fortified matrix sample and favorable results were obtained (Table 3).

Table 3. Recovery levels in 2 µg/L of spiked tap water and 2 µg/L of added laboratory fortified matrix

	MCAA		DCAA		TCAA	
	2 µg/L spiked tap water	2 µg/L laboratory fortified matrix	2 µg/L spiked tap water	2 µg/L laboratory fortified matrix	2 µg/L spiked tap water	2 µg/L laboratory fortified matrix
Blanks (tap water or ultrapure water)	0.282	NF	3.346	NF	3.603	NF
n=5 (quantitation value)	2.090	1.910	5.215	1.894	5.656	1.894
	2.116	1.885	5.363	1.902	5.584	1.954
	2.145	1.834	5.372	1.881	5.686	1.907
	2.144	1.939	5.325	1.836	5.603	1.890
	2.146	1.863	5.330	1.871	5.632	1.860
Average value	2.128	1.886	5.321	1.877	5.632	1.901
Average value minus blank value	1.846	1.886	1.975	1.877	2.029	1.901
Recovery level	92%	94%	99%	94%	101%	95%
%RSD	1.2%	2.2%	1.2%	1.4%	0.7%	1.8%

Accuracy and Precision

Five replicates were quantitated for spiked tap water samples (two concentrations) using a calibration curve created from the five tests. The average recovery level and %RSD for each set of replicates are reported in Table 4.

Table 4. Parallel test results

	MCAA	DCAA	TCAA
20 µg/L of spiked tap water			
Average concentration*	19.53	18.48	19.26
Recovery level	98%	91%	95%
%RSD	2%	1%	2%
2 µg/L of spiked tap water			
Average concentration*	1.82	1.96	2.16
Recovery level	91%	98%	108%
%RSD	4%	3%	6%

*The average concentration was calculated using the value after subtracting the blank concentration.

Conclusion

A highly sensitive LC-MS/MS method for measuring haloacetic acids using a dedicated Acclaim HAA HPLC column has been established. Under these analysis conditions, the ionization-inhibiting chloride ions, nitric acid ions, sulfuric acid ions, and haloacetic acids can be separated, making it possible to perform reliable measurements even when interfering anions are not removed using an SPE cartridge or alternative sample preparation. In addition, reproducible results were obtained for samples at concentrations more than ten times lower than regulated amounts. Accuracy and precision in tap water was confirmed in repeated testing.

Reference

1. Oral presentation 2E-5 at the 2012 Japan Society for Environmental Chemistry Symposium, “Quantification analysis of haloacetic acid based on LC-MS/MS using mixed mode columns”.

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