EPA Method 557 – Analysis of Haloacetic Acids, Dalapon, and Bromate in Drinking Water by IC-MS/MS

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Application Note 630

Key Words
Environmental analysis, ion chromatography, disinfection byproducts, haloacetic acids, HAAs, dalapon, bromate, IC-MS/MS

Goal

To demonstrate a simple and sensitive IC-MS/MS method for analyzing haloacetic acids, the pesticide dalapon, and bromate in water using EPA Method 557.

Introduction

Haloacetic acids (HAAs) are formed as disinfection byproducts when water is chlorinated to kill bacteria. Chlorine reacts with naturally occurring organic and inorganic matter in the water, such as decaying vegetation, to produce disinfection by-products (DBPs) that include HAAs. Of the nine species of HAAs, five are currently regulated by the U.S. Environmental Protection Agency (EPA) (HAA5): monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), and dibromoacetic acid (DBAA). The remaining four HAAs are currently unregulated: bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), dibromochloroacetic acid (DBCAA), and tribromoacetic acid (TBAA). However, they are also of health concern and are often analyzed along with the HAA5. This method allows for the analysis of all nine HAAs, plus bromate and the pesticide dalapon in the same IC-MS/MS run without sample preparation.

According to the EPA, there is an increased risk of cancer associated with long-term consumption of water containing levels of HAAs that exceed 0.06 mg/L (60 µg/L).[1] EPA Methods 552.1, 552.2, and 552.3, are used to determine the level of all nine HAAs in drinking water.[2-4] These methods require derivatization and multiple extraction steps followed by gas chromatography (GC) with electron capture detection (ECD).

By comparison to the conventional EPA methods using GC with ECD, the combination of ion chromatography and mass spectrometry (IC-MS and IC-MS/MS) offers sensitive and rapid detection without the need for sample pretreatment. In order to develop a simple, easy to use direct injection method, the EPA promulgated Method 557[5] for the analysis of haloacetic acids, bromate, and dalapon in drinking water by IC-MS/MS.

Experimental

Ion Chromatography

Ion chromatography analysis was performed on a Thermo Scientific™ Dionex™ ICS-5000⁺ Reagent-Free™ IC system (SP Single Pump, EG Eluent Generator, and DC Detector Column modules). Samples were directly injected and no sample pretreatment was required. The IC conditions used are shown in Table 1.

The sample was injected without cleanup or concentration onto a Thermo Scientific™ Dionex™ IonPac™ AS24 column specifically designed to separate method analytes from the following common anions (matrix components) in drinking water: chloride, carbonate, sulfate, and nitrate.

Hydroxide eluent was generated using electrolytic eluent generation, which provides accurate and precise gradients without baseline shifts. A continuously regenerated trap column continuously removes contaminants to provide pure eluent throughout the run. A Thermo Scientific™ Dionex™ AERS™ 500 suppressor was placed in line after the column that electrolytically converted hydroxide eluent into water and simultaneously removed cations present in the drinking water and eluent. See Figure 1.

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A matrix diversion valve was placed in line prior to the mass spectrometer to divert the high sample matrix anions from the MS source that normally cause signal suppression in the MS. Thus, the use of hydroxide eluent and suppression in the Reagent-Free IC system is more powerful for the separation and detection of organic acids than reversed-phase separations that require pH adjustment (to protonate to acetic acids) or addition of ion pairing agents, both of which undermine analysis. Isopropyl alcohol, at 0.2 mL/min, was added into the eluent stream via a mixing tee immediately after the matrix diversion valve. The isopropyl alcohol had two main purposes: to assist in the desolvation of the mobile phase and to act as a makeup flow when the IC eluent was diverted to waste. Acetonitrile can also be used instead of isopropyl alcohol; however, the lower cost of isopropyl alcohol is an advantage to the chemist.

Table 1. Ion chromatography system conditions.

Parameter	Value
Column	Dionex IonPac AG24 (2 x 50 mm), IonPac AS24 (2 x 250 mm), IonPac AG24A (2 x 50 mm), IonPac AS24A (2 x 250 mm)
Suppressor	Dionex AERS 500 (2 mm)
Column temperature	15 °C
Injection volume	100 µL
Flow rate	0.3 mL/min potassium hydroxide (KOH) gradient, electrolytically generated (Table 2)

Table 2. Electrolytically formed hydroxide gradient details.

Retention Time (min)	[KOH] (mM)
0.0	7.0
15.1	7.0
30.8	18.0
31	60.0
46	60.0
47	7.0
58	7.0

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer with a heated electrospray ionization (HESI-II) probe. The MS conditions used are shown in Table 3.

Table 3. Mass spectrometer source conditions.

Parameter	Value
Ion source polarity	Negative ion mode
Spray voltage	3200 V
Vaporizer gas pressure	45 units N_2
Auxiliary gas pressure	10 units N_2
Capillary temperature	200 °C
Vaporizer temperature	200 °C
Collision gas pressure	1.5 mTorr Ar
Ion cycle time	0.5 s

Individual standards were infused into the mass spectrometer to determine optimum RF lens settings and collision energies for the product ions. Table 4 describes the MS conditions for specific HAAs, dalapon, bromate, and internal standards.

Data Analysis

Data were analyzed using Thermo Scientific™ TraceFinder™ software version 3.2.

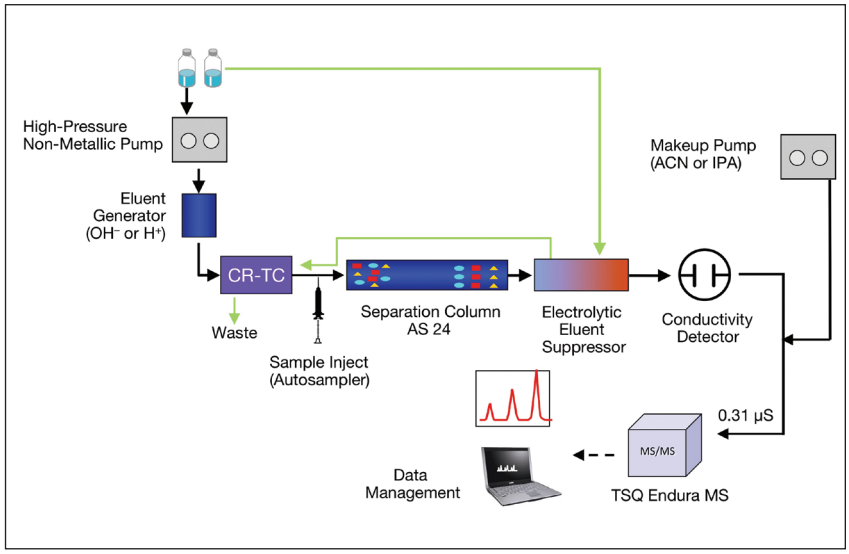

Figure 1. Flow schematic of the IC-MS/MS system.

Table 4. Optimized MS transitions for each compound analyzed in this experiment. Per the EPA method, only one product ion was monitored for each precursor ion.

Analyte	Q1 (*m/z*)	Q3 (*m/z*)	RF lens (V)	CE (V)
MCAA	92.9	35.0	67	10
MBAA	136.9	79.0	60	13
DCAA	126.9	82.9	70	10
DBAA	216.8	172.8	72	12
BCAA	172.9	128.9	70	11
TCAA	160.9	116.9	45	8
BDCAA	162.9	81.0	60	10
DBCAA	206.9	81.0	90	16
TBAA	252.8	81.0	70	17
Dalapon	140.9	96.8	56	7
Bromate	126.9	110.9	90	22
MCAA-ISTD	94.0	35.0	67	10
MBAA-ISTD	138.0	79.0	60	13
DCAA-ISTD	128.0	84.0	70	10
TCAA-ISTD	162.0	118.0	45	8

Results and Discussion

Data acquisition and processing were carried out using Thermo Scientific™ TraceFinder™ software version 3.2. The separation of the nine HAAs and two other analytes is shown in Figure 2. This chromatogram is from the laboratory synthetic sample matrix (LSSM) fortified at 20 µg/L. The LSSM is a prepared matrix of 250 mg/L of each of chloride and sulfate, 150 mg/L of bicarbonate, 20 mg/L of nitrate, and 100 mg/L ammonium chloride preservative, for a total chloride concentration of 316 mg/L. All eleven compounds are shown in Figure 2. The selectivity of the IC-MS/MS system allows separation of the HAAs from common inorganic matrix ions. This allows matrix peaks of chloride, sulfate, nitrate, and bicarbonate to be diverted to waste during the analytical run and avoids premature fouling of the ESI-MS/MS instrument source. Figure 3 shows the conductivity detector response chromatogram. The response from the Cl^-, SO_4^{2-} and NO_3^- can be seen in the trace. However, these ions do not coelute with the HAAs and they are diverted to waste using the method-controlled 6-port valve on the mass spectrometer. Specifically, the IC stream is diverted to waste from 0–12 minutes, 16–22.75 minutes, 30–37 minutes, and from 48 minutes until the end of the run.

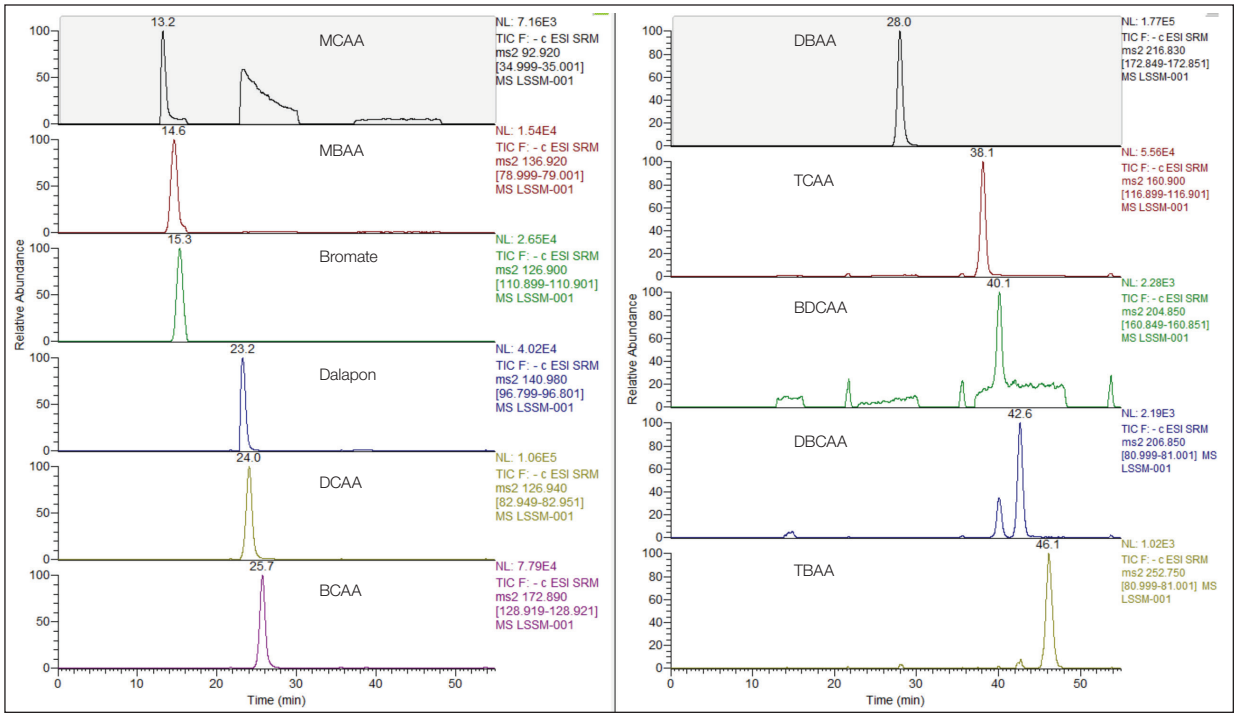

Figure 2. Overlaid chromatograms of the LSSM fortified at 20 µg/L collected on a Dionex ICS-5000$^+$ system coupled to a TSQ Endura MS.

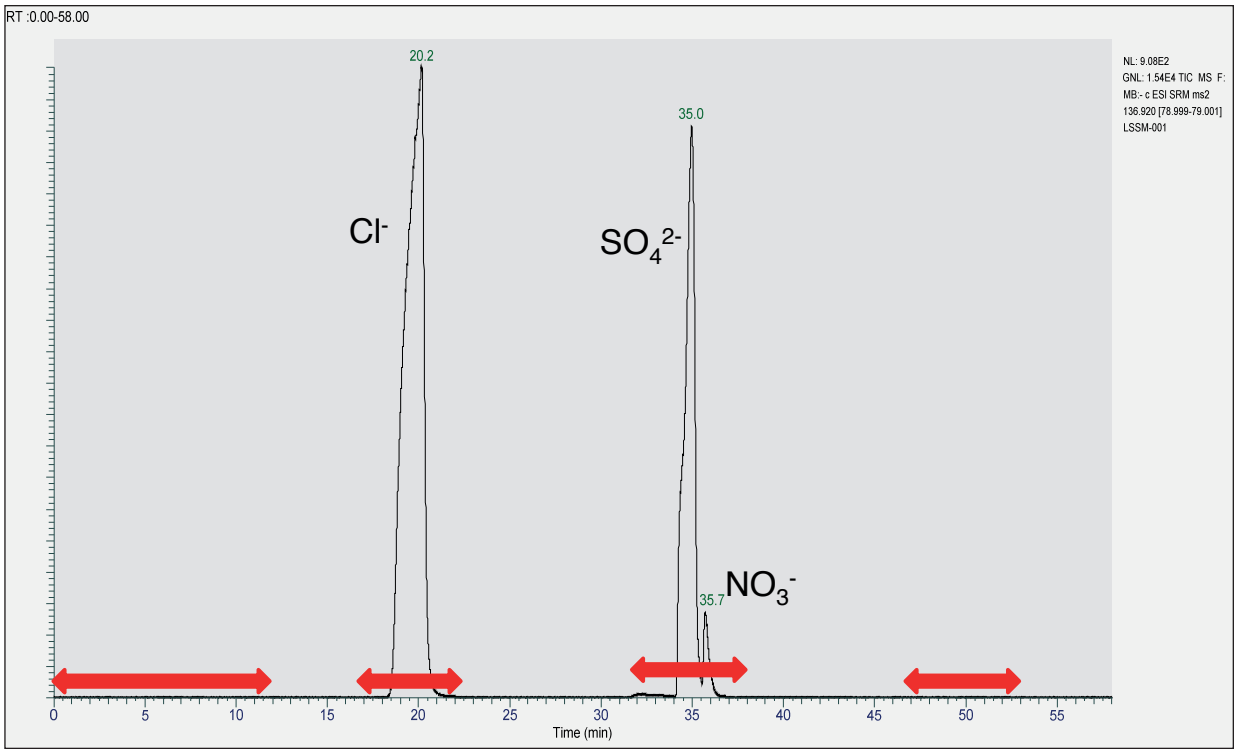

Figure 3. Conductivity detector trace showing the response of Cl^-, SO_4^{2-}, and NO_3^- added to the laboratory synthetic sample matrix. These peaks were diverted to waste and did not enter the mass spectrometer, as indicated by red arrows.

An internal standard mixture of ^{13}C-labeled MCAA, MBAA, DCAA, and TCAA was spiked into each sample at 4 µg/L. All calibration standards were prepared in deionized water containing 100 mg/L NH_4Cl as a preservative. The calibration curves were generated using internal standard calibrations for all of the HAA compounds in water. Excellent linearity results were observed for all compounds. Analytes were run at levels of 250 ng/L to 20 µg/L in a seven-point calibration curve. All of the HAAs were detected at all concentration levels (20, 10, 5, 2, 1, 0.5, and 0.25 µg/L). It should be noted that TCAA sensitivity is very strongly correlated with the source temperature of the mass spectrometer and the TBAA sensitivity decreases at higher column temperatures. Higher source temperatures caused a loss in TCAA signal. For TBAA, the column temperature was maintained at 15 °C as specified in the EPA method. Additionally, to improve the TCAA detection, the effect of temperature of the MS source on TCAA's response was tested. Temperatures of 200 °C for both the ion transfer tube and vaporizer were found to be optimal for TCCA detection without impacting the detection of the other eight analytes. This phenomenon of TCAA temperature sensitivity has been reported in studies with other MS instrumentation configurations.[6]

Method detection limits were calculated by seven replicate injections of 0.5 µg/L of each analyte and the equation $MDL=t_{99\%} \times S(n-7)$, where: t is Student's t at 99% confidence intervals ($t_{99\%}$, n=7 = 3.143) and S is the standard deviation. Table 6 compares these results to the calculated MDL values of EPA Method 552.2, which uses liquid-liquid extraction and methylation of the carboxylic acids before determination by GC-ECD. The results obtained by the IC-MS/MS method exceeded the MDLs reported in EPA Method 552.2, with the exception of DBCAA.

Table 6. MDL calculation results for each compound for seven replicate injections of the 0.5 µg/L calibration standard compared to the MDLs from EPA Method 552.2. Note the EPA method calculated the MDLs on various fortified levels. Please refer to the EPA method for details.

Analyte	Calculated MDL (µg/L)	EPA Method 552.2 MDL (µg/L)	%RSDs (n=7)
MCAA	0.105	0.273	10.7
MBAA	0.104	0.204	6.3
DCAA	0.044	0.242	2.8
DBAA	0.021	0.066	1.6
BCAA	0.059	0.251	4.4
TCAA	0.033	0.079	3.0
BDCAA	0.141	0.091	10.6
DBCAA	0.214	0.468	15.9
TBAA	0.159	0.820	15.6
Dalapon	0.050	N/A	3.7
Bromate	0.059	N/A	6.3

Municipal Drinking Water Sample Analysis

Additionally, municipal drinking water from San Jose, CA, was analyzed for the presence of any of the analytes contained in the method. Municipal drinking water samples were collected in accordance with the procedure for EPA Method 557, with NH_4Cl added as a preservative, as it reacts with residual chlorine preventing further formation of haloacetic acids. Internal standards were added and the samples were quantified. The levels of each compound detected in the samples are shown in Table 7. The total amount of haloacetic acids for all nine HAAs was 35.62 µg/L. For the regulated HAA5, the total was 30.21 µg/L. The MCL set by the US EPA for the HAA5 is 0.060 mg/L(60 µg/L).[7] This sample was below that limit, at 0.03021 mg/L (30.21 µg/L).

Table 7. Results of analysis of San Jose, CA, municipal drinking water for the presence of HAAs. Eight out of the nine HAAs analyzed plus bromate were detected. The total amount of HAAs present in this sample was 35.62 µg/L.

Analyte	Detected Amount (µg/L)
MCAA	1.08
MBAA	N/D
DCAA	15.21
DBAA	0.95
BCAA	2.65
TCAA	12.97
BDCAA	2.07
DBCAA	0.69
TBAA	N/D
Dalapon	N/D
Bromate	0.44

Analysis of Five Regulated HAAs

If the entire panel of nine haloacetic acids is not required, the runtime can be shortened to 45 minutes. The adjusted gradient is shown in Table 8. All other parameters are identical to those used in the analysis of the nine HAAs. Additionally, the AG24A guard column and AS24A analytical column can be utilized. Retention times for the HAAs will not change; however, the AS24A column offers superior separation of the chlorite ion that may co-elute with MCAA. An example chromatogram showing the separation of the five HAAs is shown in Figure 4.

Table 8. Electrolytically formed hydroxide gradient for the analysis of five HAAs.

Time (min)	[KOH] (mM)
0.0	7.0
15.1	7.0
30.8	18.0
31	60.0
40	60.0
40.1	7.0
45	7.0

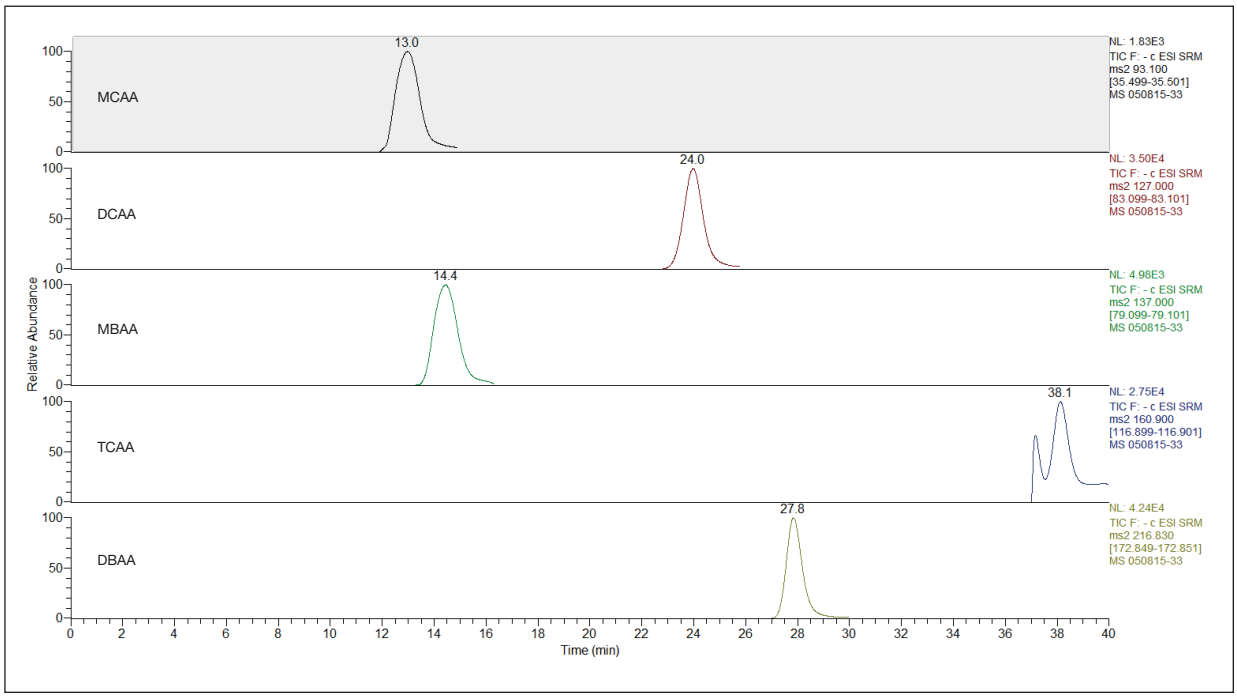

Figure 4. Extracted MS chromatograms of the five HAAs regulated by the US EPA. LSSM fortified sample at 2 µg/L collected on a Dionex ICS-5000⁺ system coupled to a TSQ Endura MS. Note, the descending baseline seen just before the TCAA peak is due to the divert valve switching and is not a response from TCAA.

Conclusion

Reagent Free IC systems coupled with an MS/MS detector are a powerful tool used in the quantitation of haloacetic acid samples. When compared to the conventional EPA methods using GC with electron capture, using the combination of the ICS-5000⁺ ion chromatography system and the TSQ Endura triple quadrupole mass spectrometer to analyze for haloacetic acids saves analysts several hours of sample preparation as well as eliminating costly and toxic derivitization agents. This time savings is a result of the lack of time-consuming sample pretreatment while offering sufficient sensitivity to quantitate all nine haloacetic acids. The resolution between the matrix peaks and haloacetic acids is excellent, which allows for minimum interference in detection, as well as ensuring a cleaner ion source of the mass spectrometer.

Excellent reproducibility and quantitation of HAAs was achieved when samples were spiked into a simulated matrix containing 250 mg/L of each of chloride and sulfate, 150 mg/L bicarbonate, 20 mg/L of nitrate, and 100 mg/L ammonium chloride preservative for a total chloride concentration of 316 mg/L. Results are better than those achieved in EPA Method 552.2, except for BDCAA, and comparable to those in EPA Method 557, except for higher MDLs for brominated compounds.

Acknowledgments

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