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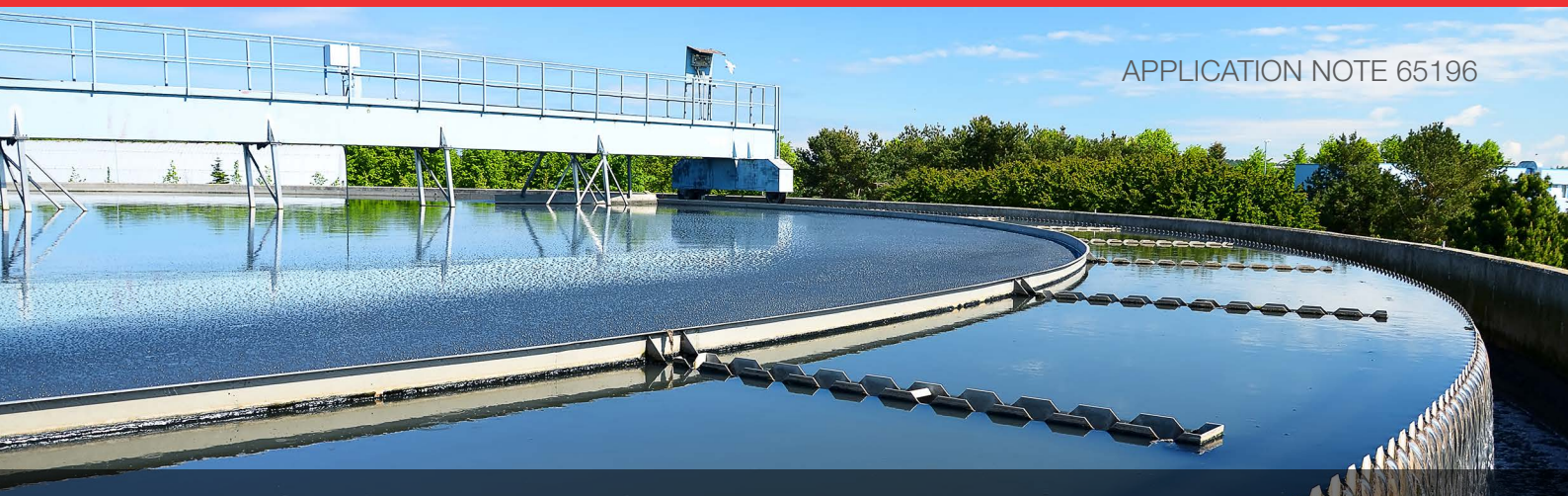

APPLICATION NOTE 65196

Tomorrow's quantitation with the TSQ Fortis mass spectrometer: robust, reproducible quantitation workflows of haloacetic acids, bromate, and dalapon in water according to EPA Method 557

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Keywords

Haloacetic acids, bromate, dalapon, triple quadrupole MS, TSQ Fortis MS, quantitation workflow solution, disinfection byproducts, TraceFinder software, EPA Method 557

Goal

Development and easy implementation of a robust, reliable, and reproducible workflow solution for the analysis and quantitation of nine haloacetic acids, bromate, and dalapon in water using a triple quadrupole mass spectrometer (MS).

Application benefits

- Development of a robust workflow for analysis and quantitation of haloacetic acids in water with ion chromatography (IC) and Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer (QqQ)
- Leveraging enhanced performance of a robust QqQ with the required sensitivity to address critical analytical challenges in environmental safety while reducing cost/sample

Introduction

Clean drinking water is becoming more scarce in today's world and contamination can result in long-lasting damage to human health. Along with purifying water by means of mechanical measures, disinfection also plays an essential role in ensuring the supply of clean drinking water. Drinking water goes through an extensive disinfection process to ensure high quality;

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however, by-products from the disinfection process can result in health risks. As an example, haloacetic acids (HAAs) form as a result of the disinfection of by-products when water is chlorinated to kill disease-causing microbes.[1] Bromate is formed when disinfecting ozone reacts with naturally occuring bromide. Regardless of how these by-products form, excessive consumption can result in serious health issues, such as cancer.[2]

As described above, HAAs are formed as a result of chlorination of water where chlorine reacts with naturally occurring organic and inorganic matter in the water, such as decaying vegetation, to produce disinfection by-products (DBPs), including HAAs. Of the nine species of HAAs, five are currently regulated by the 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). Bromate can arise as a by-product of the ozonation of bromide-containing water depending on the conditions (pH, temperature, etc.) prevalent at the treatment site.[3] According to regulations, drinking water plants must determine the concentration of disinfection by-products in drinking water prior to release. EPA Method 557 has been validated for the determination of haloacetic acids, bromate, and dalapon.

The analysis of contaminants, especially polar molecules in drinking water, can be effected using one of several techniques. Analysis of polar molecules utilizing LC is challenging, as LC typically works best for non-polar molecules, and suffers from high matrix resulting from groundwaters that are often evaluated prior to entry into drinking water utilities. This calls for derivatization of samples, which can be time consuming and adds challenges towards achieving the result, faster and with confidence. Fortunately, ion chromatography (IC) offers some significant benefits owing to its capability to analyze polar molecules, especially in higher matrix waters. In this study, a robust, reliable, reproducible quantitation assay for determination of HAAs, bromate, and dalapon in drinking water with IC-MS/MS using a Thermo Scientific™ Dionex™ ICS-5000⁺ Hybrid HPIC™ system, a Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer, and Thermo Scientific™ TraceFinder™ version 4.1 software is reported.

Experimental

Sample preparation

Drinking water samples were collected from municipal tap water sources. NH_4Cl was added as a preservative at 100 mg/L to all water samples. No further sample preparation was performed prior to injection.

Ion chromatography

IC analysis was performed on the Dionex ICS-5000⁺ Hybrid HPIC system. Samples were directly injected; no sample pre-treatment was required. The IC KOH gradient conditions are indicated in Table 1. A 100 µL sample was injected onto a 2 × 250 mm Thermo Scientific™ Dionex™ IonPac™ AS24A column, which is specifically designed to separate method analytes from the following common anions (matrix components) in drinking water: chloride, carbonate, sulfate, and nitrate. A guard column (Thermo Scientific™ Dionex™ IonPac™ AG24A, 2 × 50 mm column) and a Thermo Scientific™ Dionex™ ASRS-500 electrolytically regenerated suppressor were used. The mobile phase was 300 µL/min KOH, which was automatically prepared by the eluent generator of the ICS-5000⁺. The concentration of the KOH was changed during the method run to achieve a gradient elution profile. Isopropyl alcohol was added to the eluent post column via a T at a rate of 200 µL/min to assist in nebulization of the eluent in the MS ion source. The Thermo Scientific™ Dionex™ AXP auxiliary pump water for suppressor regeneration was maintained at 600 µL/min. The column temperature was maintained at 15 °C.

Table 1. IC gradient information

Time (min)	KOH Concentration (mM)
0.00	7.00
15.10	7.00
30.80	18.00
31.00	60.00
46.00	60.00
47.00	7.00
58.00	7.00

Hydroxide eluent was generated using an electrolytic eluent generation, which provides smoother and more reproducible gradients than conventional pump proportioning valves, and a continuously regenerated trap column removed contaminants to provide pure eluent throughout the run. A matrix diversion valve was placed in line prior to the mass spectrometer (MS) to divert the high sample matrix anions from the mass spectrometer source that normally cause signal suppression in the mass spectrometer. 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 acidic addition (to protonate the compounds to acetic acids) or addition of stabilizing salts, both of which undermine analysis. Isopropyl alcohol (0.2 mL/min) was added into the eluent stream via a mixing tee immediately after the matrix diversion valve. The isopropyl alcohol enabled desolvation of the mobile phase and acted as a makeup flow when the IC eluent was diverted to waste.

Mass spectrometry

The TSQ Fortis triple quadrupole mass spectrometer was used for this analysis. All compounds for this study were analyzed in negative ion heated electrospray (HESI) mode. The experimental conditions were optimized with a static spray voltage, a cycle time of 2.3 s, and both Q1 and Q3 resolution were maintained at 0.7 Da FWHM. The SRM table along with other critical MS features for all the target analytes are listed in Table 2.

Individual standards were infused into the mass spectrometer to determine optimum tube lens settings and collision energies for the product ions.

Software

Data acquisition and processing were conducted using TraceFinder software version 4.1

Table 2. Optimized mass spectrometer transitions for each compound analyzed in this experiment. Following EPA Method 557,[4] only one product ion was monitored for each precursor ion.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	Tube Lens (V)	Source Fragmentation (V)
MCAA	92.85	35.1	10.23	92	22.9
MCAA_IS	93.99	35.1	10.23	92	22.9
DCAA	127.00	83.0	10.23	57	0
Bromate	127.00	110.8	21.56	68	13.1
MBAA	136.85	78.9	11.82	45	0
MBAA_IS	137.94	78.9	10.23	52	0
DCAA_IS	128.00	84.0	10.23	50	0
Dalapon	141.00	97.0	10.23	53	0
TCAA_161	160.81	116.9	10.23	55	0
TCAA_IS	161.91	117.8	10.23	42	0
BDCAA	163.00	81.0	10.23	63	21.2
TCAA_163	163.00	119.0	10.23	56	27.7
BCAA	172.77	128.8	10.23	73	0
DBCAA	207.00	79.0	14.55	82	18
DBAA	216.78	172.7	10.23	58	0
TBAA	251.00	79.0	18.64	84	21.2

Results and discussion

The data obtained were from the laboratory synthetic sample matrix (LSSM). The LSSM is a prepared matrix of 250 mg/L 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. Chromatograms of all eleven compounds are shown in Figure 1. The selectivity offered by the Dionex IonPac AS24A column enabled good separation of the HAAs from the typical inorganic matrix ions. Such selectivity and ability to resolve and identify every analyte signal allows matrix signals of chloride, sulfate, nitrate, and bicarbonate to be diverted to waste during the analytical run and avoids contamination of the ESI-MS/MS instrument source. This capability is not possible with LC-based separations.

An internal standard mixture of ^{13}C-labeled MCAA, MBAA, DCAA, and TCAA was spiked into each sample at 4 ppb. The chromatograms of each of the ^{13}C-labeled analytes at 4 µg/L are shown in Figure 2. 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 the HAAs in water.

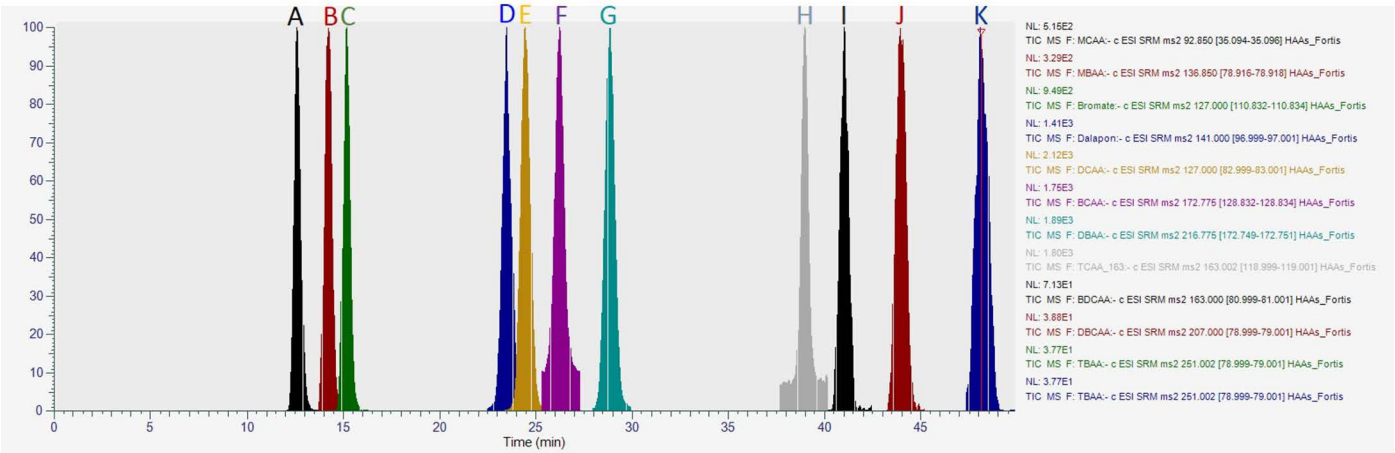

Figure 1. Ion chromatograms of HAAs: (A) MCAA, (B) MBAA, (C) Bromate, (D) Dalapon, (E) DCAA, (F) BCAA, (G) DBAA, (H) TCAA, (I) BDCAA, (J) DBCAA and (K) TBAA at 1 µg/L

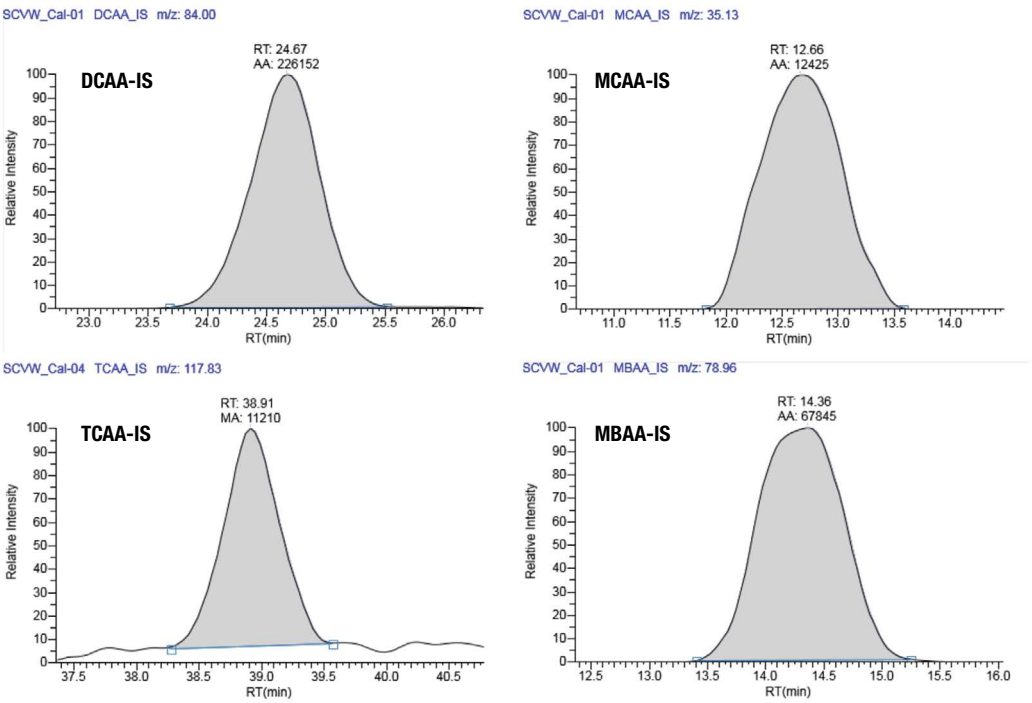

Figure 2. Ion chromatograms of the internal standards of the four mentioned analytes at 4 µg/L

Linearity greater than 0.99 was achieved for all 11 components observed, and each of the analytes were run over the entire concentration range in a six-point calibration curve. HAAs were calibrated between the range of 0.25 µg/L to 20 µg/L, exhibiting two orders of linear dynamic range (Figure 3).

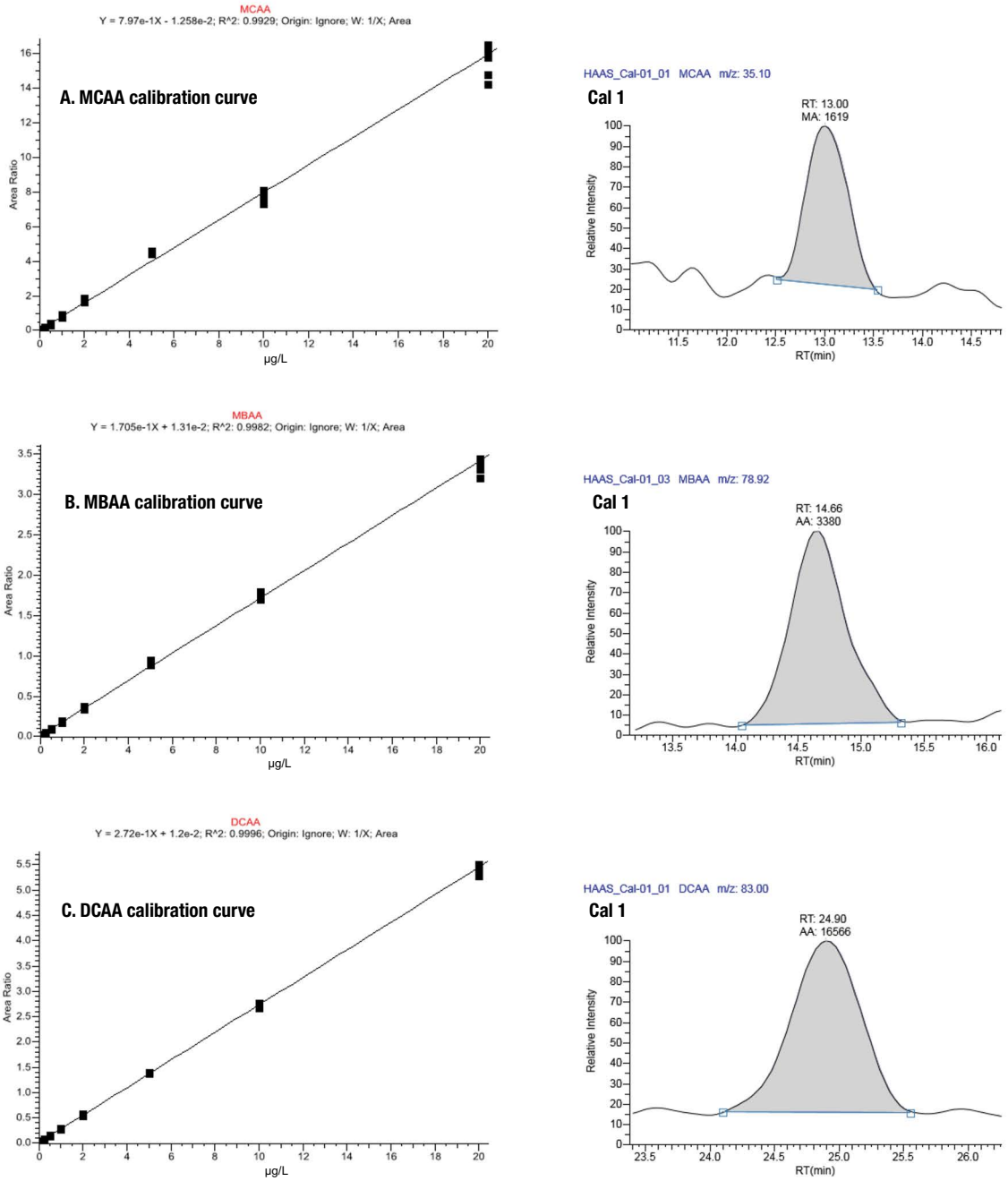

Figure 3-1. Calibration curve with the chromatogram at the lowest concentration calibrator (0.25 µg/L) for each HAA: (A) MCAA, (B) MBAA, (C) DCAA

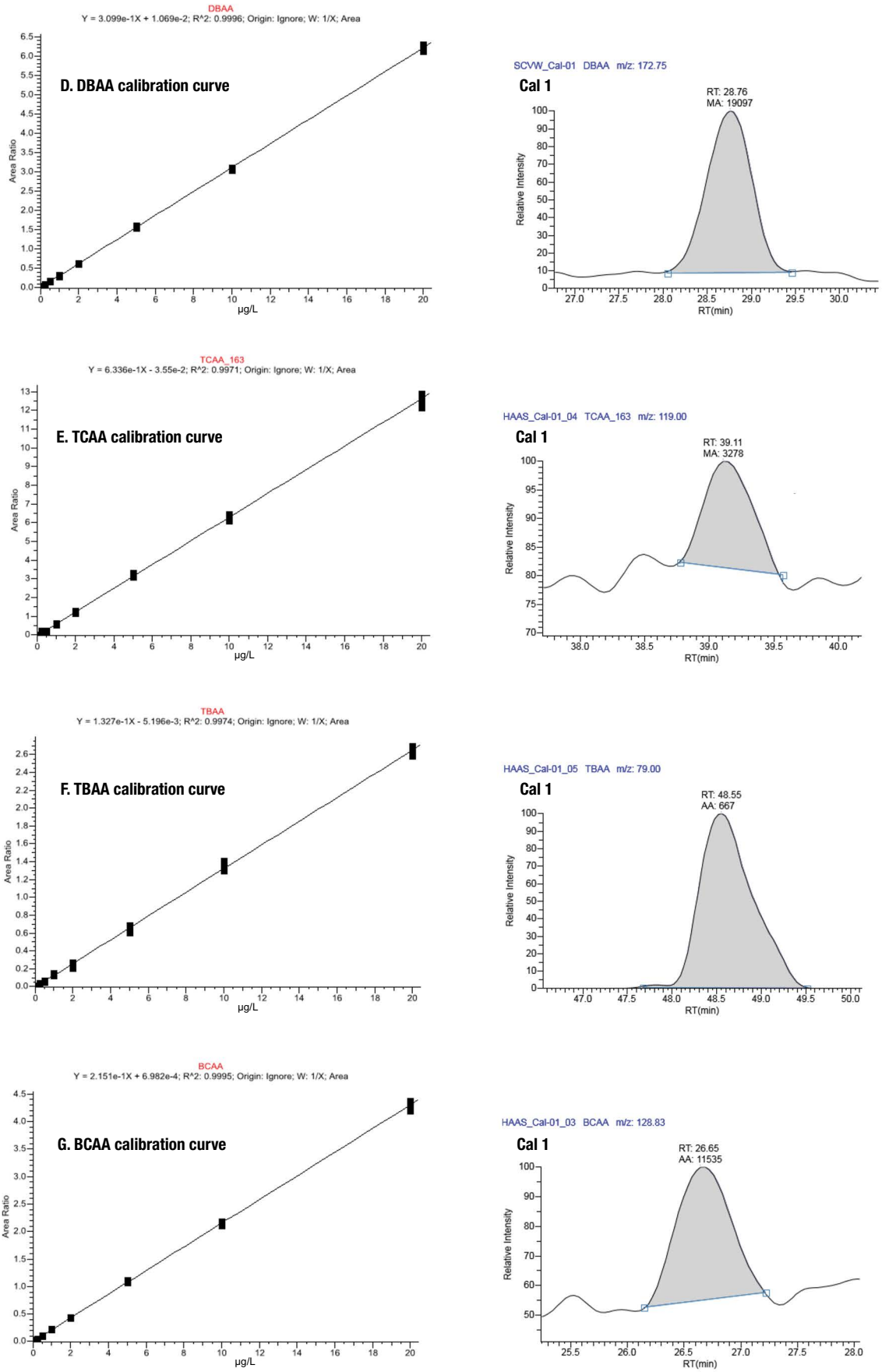

Figure 3-2. Calibration curve with the chromatogram at the lowest concentration calibrator (0.25 µg/L) for each HAA: (D) DBAA, (E) TCAA, (F) TBAA, (G) BCAA

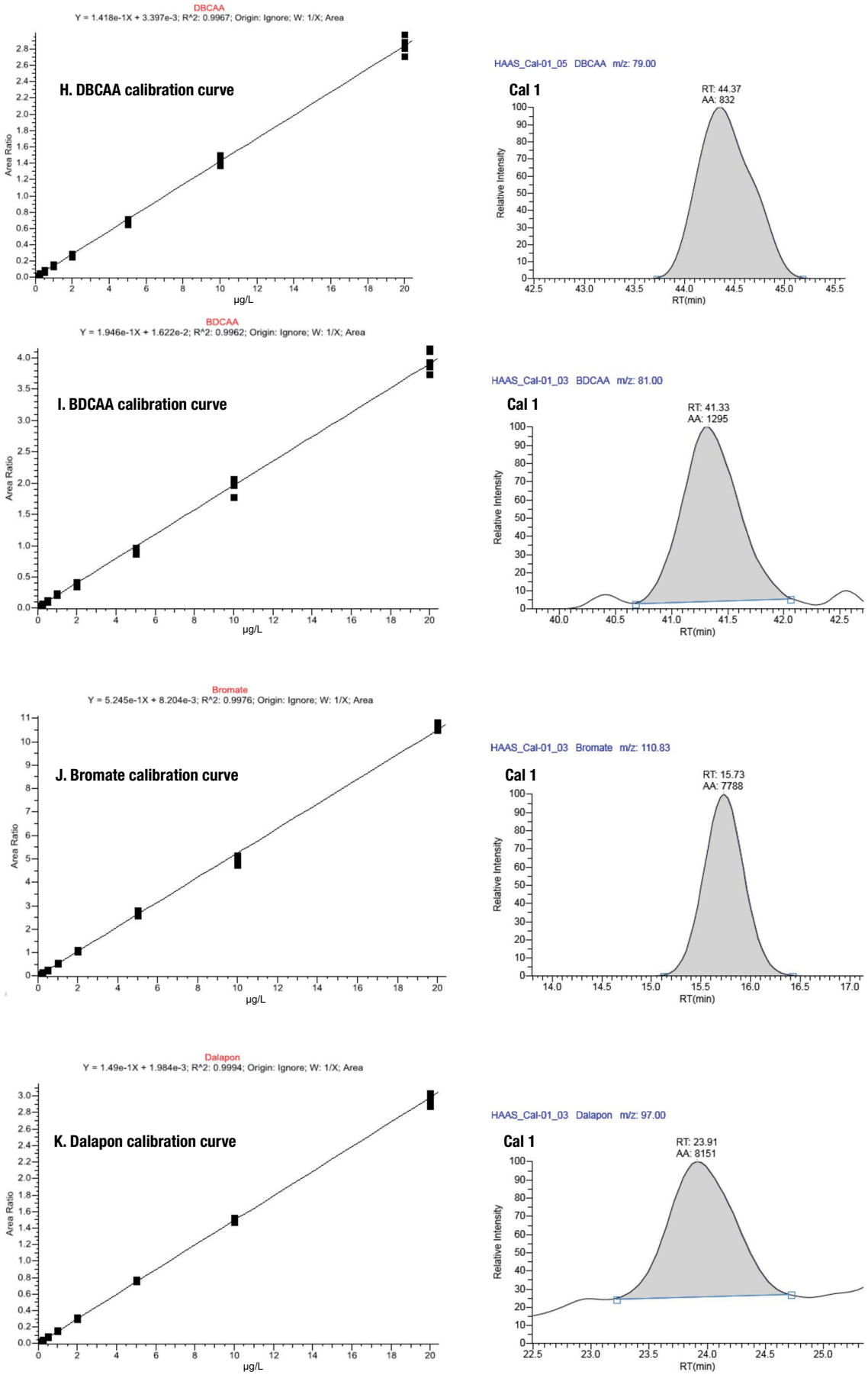

Figure 3-3. Calibration curve with the chromatogram at the lowest concentration calibrator (0.25 µg/L) for each HAA: (H) DBCAA, (I) BDCAA, (J) Bromate, and (K) Dalpon

All the HAAs were detected at all concentration levels (Figure 3 and Table 3). Some of the analytes, such as MCAA, TBAA, DBCAA, and BDCAA, had responses that approached their limits of detection at 0.25 µg/L. However, the workflow solution utilizing the Dionex ICS-5000+ Hybrid HPIC system and TSQ Fortis MS allows enough sensitivity, selectivity, and robustness to detect each of the HAAs at all concentration ranges. In addition, it should be noted that TCAA sensitivity is very strongly correlated with the source temperature of the mass spectrometer as well as the column temperature of the IC column. For this reason, 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 the response of TCAA was tested. Temperatures of 200 °C for both the ion transfer tube and vaporizer were found to be optimal for TCAA 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 and also has an effect on brominated HAAs.[4]

Tap water sample analysis

Tap water samples from different cities in the Bay Area, were analyzed for the presence of all analytes contained in the method. Tap water samples were collected in accordance with the EPA Method 557 procedure,[5] with NH_4Cl added as a preservative as it reacts with residual chlorine preventing further production of haloacetic acids after sampling. Internal standards were added and the samples were quantified. The levels of each compound detected in the samples are shown in Table 4. The amount of HAA5 (MCAA, DCAA, TCAA, MBAA, and MCAA) is less than the maximum contaminant level, 60 µg/L.

Table 3. Peak area for each HAA over the concentration range (0.25–20 µg/L)

Conc. (µg/L)	MCAA	MBAA	DCAA	DBAA	TCAA	TBAA	BCAA	DBCAA	BDCAA	Bromate	Dalapon
0.25	1427	3209	17596	19525	2905	608	11483	853	1380	7967	8472
0.5	2879	5691	33336	37950	5024	1412	23423	1742	2671	15759	17263
1	6641	10458	66049	73734	14692	3285	51086	3487	5255	31539	35453
2	13815	20473	132264	147128	31505	6245	102765	6999	9581	61106	72283
5	29513	42485	273500	307224	67865	13724	213529	14369	19763	126251	148971
10	57913	91405	555573	628113	132877	28156	436224	30277	41249	260205	303844
20	109508	167859	1099224	1257242	265959	56145	865828	60456	83566	525701	599003

Table 4. Detected concentrations of the compounds

Compound	LSSM (µg/L)	MRL (µg/L)	QCS (µg/L)	City A (µg/L)	City A LFM* (µg/L)	City A LFMD* (µg/L)
MCAA	9.04	0.42	4.0	0.83	2.80	2.76
MBAA	10.16	0.51	5.2	0.54	2.60	2.51
Bromate	9.95	0.47	5.3	0.00	2.23	2.14
DCAA	10.29	0.51	7.6	5.78	7.77	7.69
Dalapon	10.35	0.58	–	0.21	2.36	2.24
BCAA	10.45	0.55	9.7	5.10	6.72	6.72
DBAA	10.23	0.55	5.5	2.63	4.38	4.28
TCAA	9.81	0.27	1.2	4.31	6.07	6.21
TBAA	9.96	0.55	–	0.63	2.48	2.60
BDCAA	9.82	0.59	–	6.03	7.98	7.90
CDBAA	9.87	0.58	–	3.83	5.34	5.84

LFM = Laboratory Fortified Matrix
LFMD = Laboratory Fortified Matrix Duplicate

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Conclusion

The presence of disinfectants ensures increased safety for drinking water; however, the by-products of disinfectants also give rise to HAAs, bromates, and dalapon, excessive consumption of which can result in severe health issues. Analysis and quantitation of these contaminants in water can pose several challenges, especially with the increasing complexity of contaminants. Reagent-free IC systems coupled with the TSQ Fortis MS is a powerful platform solution that offers several advantages towards developing robust, reproducible, fast, and sensitive quantitation of polar molecules, as shown in this report. A robust, reproducible workflow solution for the analysis and quantitation of HAAs, bromates, and dalapon was developed. This method offers significant advantages over GC-ECD methods such as EPA Method 552 that require up to 4 hrs of sample preparation per sample. This IC-MS/MS method is direct injection and requires no sample preparation, thus offering significant advantages and cost savings.

All the analytes in this assay were detected to the lowest calibration level and the accuracy is within the criteria. All 22 samples that were tested against a previously provided calibration curve achieved higher sensitivity with better robustness. The resolution between the matrix peaks and HAAs is excellent, which allows for minimum interference in detection, as well as ensuring a cleaner ion source of the mass spectrometer. Last but not the least, the optimal performance of the Dionex ICS-5000^{+} Hybrid HPIC system and TSQ Fortis MS platform solution exhibited excellent reproducibility and quantitation of the HAAs in water samples.

References

1. Beck, J. R., et al. "EPA Method 557 Quantitation of Haloacetic Acids, Bromate, and Dalapon in Drinking Water Using Ion Chromatography and Tandem Mass Spectrometry." Thermo Fisher Scientific Poster Note 64430, **2016.**
2. http://water.epa.gov/drink/contaminants/basicinformation/dalapon.cfm.
3. Pisarenko, A. N., et al. Rapid analysis of perchlorate, chlorate and bromate ions in concentrated sodium hypochlorite solutions. *Analytica Chimica Acta,* **2010,** *659*(1-2), 8.
4. Slignsby, R.; Saini, C.; Pohl, C.; Jack, R. The Measurement of Haloacetic Acids in Drinking Water Using IC-MS/MS–Method Performance, Presented at the Pittsburgh Conference, New Orleans, LA, March **2008.**
5. U.S. EPA Method 557: Determination of Haloacetic Acids Bromate and Dalapon in Drinking Water by Ion Chromatography Electrospray Ionization Tandem Mass Spectrometry (IC-ESI-MS/MS), 2009.

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