Extending the Quantitative Performance for Haloacetic acids, Bromate, and Dalapon in Water using an IC-MS/MS Workflow

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ABSTRACT

Purpose: To develop and implement a robust, reliable, and reproducible workflow solution for analysis and quantitation of nine haloacetic acids, bromate, and dalapon in water using ion chromatography coupled to a triple quadrupole MS.

Methods: Previous work demonstrated significant improvements for the routine quantitation of nine HAAs using a direct injection method on a Thermo Scientific™ Dionex™ ICS-5000 system, Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer, and Thermo Scientific™ TraceFinder™ 4.1 software. The workflow presented here utilizes the latest Thermo Scientific instrumentation to deliver enhanced productivity and sensitivity for current HAA analysis as well as future-proofing for tomorrow's requirements. The new method is based upon the requirements outlined in USEPA Method 557.

Results: The TSQ Fortis Plus mass spectrometer enables direct method transfer from the original workflow without requiring method optimization, eliminating disruption in data acquisition. It contains an enhanced active collision cell (Q2) that increases the transmission efficiency for low-mass product ions by an average of two-fold, resulting in improved LLOQs that are significantly below the MRLs of all HAAs. The improved LLOQs enable reduced sample loading amounts from 100 µL, as previously defined, to 50 µL while still satisfying regulatory requirements.

INTRODUCTION

Clean drinking water is becoming scarcer, and any contamination can result in long lasting damage to human health. Strategies used to purify water include mechanical measures and disinfection, which utilizes chlorination to remove microbial content.[1] The disinfection process, however, can introduce by-products that can result in health risks. The primary class of compounds associated with drinking water contamination is disinfection by-products (DBPs).[2] Haloacetic acids (HAAs) are a subgroup of DBPs,[3] which are specifically linked to cancer and other issues.

Continued research focused on identifying more potentially harmful compounds requires analytical workflows to be responsive to new requirements determining DBP concentration levels prior to release. Standard analytical methods coupling liquid chromatography to triple quadrupole mass spectrometers to perform selected-reaction monitoring (SRM) are the preferred methods due to the selectivity, sensitivity, and analysis speed. Traditional separation methods using liquid chromatography (LC) often require costly derivatization to effectively separate target compounds prior to ionization and analysis. Ion chromatography (IC), however, effectively and efficiently retains and separates the polar compounds, improving the overall IC-MS/MS workflow to meet current regulations as well as maximize workflow robustness.

MATERIALS AND METHODS

Sample Preparation

Reagent water samples were spiked with the target analyte mixtures at known amounts. Ammonium chloride (NH4Cl) was added as a preservative at 100 mg/L to all samples. No further sample preparation was performed prior to injection.

Ion Chromatography

IC analysis was performed on a Dionex ICS-6000 system. The mobile phase was 300 µL/min KOH, which was automatically prepared by the eluent generator of the ICS-6000 system. The IC KOH gradient conditions are indicated in Table 1. Isopropyl alcohol was added to the eluent post column via a tee at a flow of 300 µL/min to improve desolvation and help analyte ionization. A 50 µL sample was injected onto a 2 x 250 mm Thermo Scientific™ Dionex™ IonPac™ AS31 RFIC analytical 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 Dionex IonPac AG31, 2 ´ 50 mm guard column and Thermo Scientific™ Dionex™ ADRS 600 2 mm conductivity suppressor were used. A Thermo Scientific™ Dionex™ AXP auxiliary pump with water for suppressor regeneration was maintained at 600 µL/min. The column temperature was maintained at 15 ° C.

Time (Minutes)	KOH Concentration (mV)
0	17
5	17
7	85
18	85
40	85
40	17

Table 1. IC gradient information used for the analysis of HAAs in water

MATERIALS AND METHODS- cont.

Ion Chromatography

Hydroxide eluent is generated using an electrolytic eluent generation, which provides smoother gradients than conventional pump proportioning valves, and a continuously regenerated trap column removes contaminants to provide pure eluent throughout the run. 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 acidic addition (to protonate the compounds to acetic acids) or addition of stabilizing salts, both of which undermine analysis. Isopropyl alcohol (0.3 mL/min) was added into the eluent stream via a mixing tee immediately after the matrix diversion valve. The isopropyl alcohol enables desolvation of the mobile phase and acts as a makeup flow when the IC eluent is diverted to waste. See Figure 1 for a schematic of the components in the system.

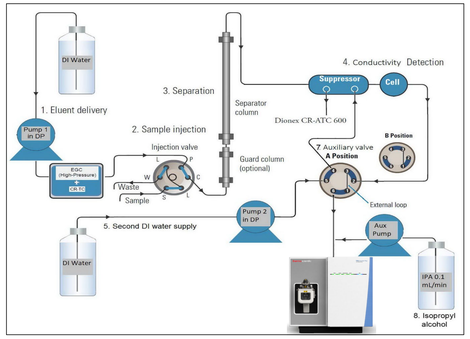


Figure 1: IC-MS/MS Schematics for Disinfection Byproducts Application Workflow

Mass Spectrometry

The TSQ Fortis Plus mass spectrometer was used for this analysis with negative heated electrospray ionization (HESI) mode. The experimental conditions were optimized with a static spray voltage of 3200 V and both Q1 and Q3 resolutions maintained at 0.7 Da FWHM. The ion transfer tube and vaporizer temperatures were maintained at 225 C and 275 C, Data acquisition and processing were conducted using Thermo Scientific™ TraceFinder™ software version 5.1. The SRM table and other critical MS features for all target analytes are listed in Table 2.

Compound	Retention Time (min)	RT Window (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Tube Lens (V)	Source Fragmentation
MCAA	6.27	6	92.912	35.1	10	79	14.6
MCAA_IS	6.27	6	93.9	35.1	9.8	82	13.1
MBAA	6.95	6	136.862	78.971	10	86	9.8
MBAA_IS	6.95	6	137.9	78.971	10	84	14.7
Bromate-79	7.4	5	126.9	110	21.68	85	24.5
Dalapon	11.35	6	140.862	96.946	7.7	84	13
DCAA	12.2	6	126.862	83	10	86	24.5
DCAA_IS	12.2	6	128	83.929	10	84	13.1
BCAA	13.15	6	172.8	128.925	11	89	22.8
DBAA	14.45	6	216.75	172.754	12	87	14.7
DBCAA_206	22	14	206.762	78.929	14	91	22.9
TCAA_IS	20	8	161.9	117.925	7	78	18
TCAA_163	20	8	162.812	118.929	5.25	79	22.9
TBAA_250	24	10	250.712	78.929	19.41	87	26.1
BDCAA	24.2	8	162.812	80.929	7.1	79	22.9

Table 2: Optimized MS transitions for each compound analyzed in this experiment. Following the U.S. EPA method, only one product ion was monitored for each precursor ion.

RESULTS AND DISCUSSION

The system was checked using a laboratory synthetic sample matrix (LSSM) as described in U.S. EPA Method 557.7. 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. The selectivity of the ion exchange column enabled good separation of the HAAs from the typical inorganic matrix ions and enabled incorporation of a post-column divert valve used to send the matrix contaminants to waste during the analytical run, improving the workflow robustness and maximizing IC-MS/MS sensitivity. Figure 2 shows the measured response for the 10 ppb (µg/L) calibration standard, demonstrating excellent separation and sensitivity.

An internal standard mixture of 13C-labeled MCAA, MBAA, DCAA, and TCAA was spiked into each sample at 4 µg/L (ppb). The chromatograms of each of the 13C-labeled analytes at their lowest quantifiable concentration are shown in Figure 2. Peak area RSD values across more than fifty replicates were less than 5% for each with excellent chromatographic peak shape.

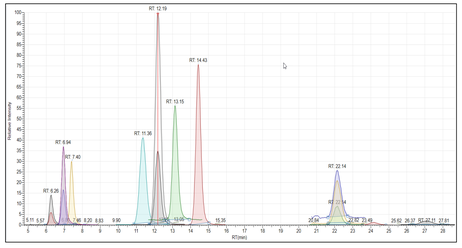


Figure 2: Extracted ion chromatogram of all eleven compounds and the internal standards at 10 ppb. A divert valve effectivity removes common anions found in water sources. Excellent specificity and chromatographic peak shape ensure accurate quantitation.

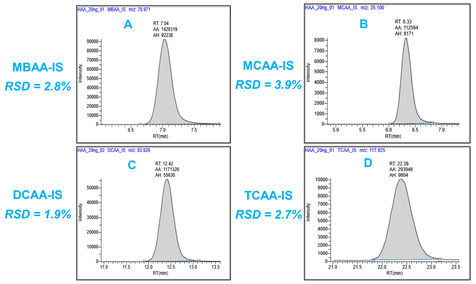


Figure 3: Internal standards and ion chromatograms of the four analytes at 4 µg/L concentration. (A) MBAA, (B) MCAA, (C) DCAA, and (D) TCAA.

Calibration and LLOQ/MDL

The results from the nine-point calibration curve covering a spiked range of 0.0625 to 20 µg/L showed outstanding linearity. Regression values greater than 0.995 were measured for all analytes over the calibration range. Tables 3A and 3B show average peak areas and reproducibility, respectively, at each calibration level for the eleven compounds. The lower limit of quantitation (LLOQ) and method detection limits (MDLs) are summarized in Table 3. Seven replicate injections were analyzed from 0.0625 to 1 µg/L and three replicate injections from 2 to 20 µg/L calibration points. Eight of the eleven compounds showed a four-fold lower LLOQ and MDL than those reported in the U.S. EPA method, while TCAA and TBAA were two-fold lower and DBCAA showed equivalent response.

RESULTS- cont.

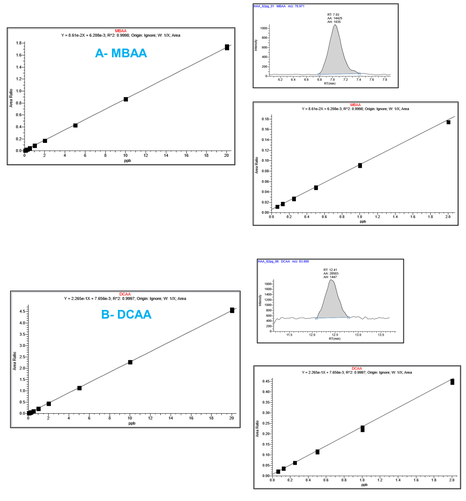
Calibration and LLOQ/MDL

The workflow solution exceeds the U.S. EPA method requirements, demonstrating excellent sensitivity and selectivity, as shown in Table 3.

Compound	EPA LLOQ (µg/L)	TSQ Fortis Plus LLOQ (µg/L)	EPA 557 Method MDL (µg/L)	TSQ Fortis Plus MDL (µg/L)
BCAA	0.25	0.0625	0.11	0.01
BDCAA	0.25	0.0625	0.05	0.02
Bromate	0.25	0.0625	0.02	0.01
Dalapon	0.25	0.0625	0.04	0.01
DBAA	0.25	0.0625	0.02	0.01
DBCAA-206	0.25	0.25	0.04	0.05
DCAA	0.25	0.0625	0.06	0.01
MBAA	0.25	0.0625	0.06	0.01
MCAA	0.25	0.0625	0.2	0.01
TBAA-250	0.25	0.25	0.07	0.05
TCAA-163	0.25	0.125	0.09	0.02

Table 3. TSQ Fortis Plus and EPA 557 method comparison of LLOQ and MDL- 7 reps in reagent water

Example calibration curves for select HAAs, bromate, and dalpon are presented in Figure 4(A-D). Two calibration curves are shown for each compound, the entire CAL range and an expanded view of the lower concentration range for each curve. The extracted ion chromatogram at the LLOQ for each component is also displayed. Excellent peak area RSDs (<15%) and calculated concentrations (±20%) of each level against the calibration curves pass the method accuracy and precision criteria. 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 temperature of the IC column. For this reason, the column temperature was maintained at 15 C as specified in the U.S. EPA method. Additionally, to improve the TCAA detection, the effect of MS source temperature was tested. Temperatures of 225 C and 275 C for the ion transfer tube and vaporizer, respectively, were found to be optimal for TCCA detection without impacting the detection of the other analytes.



RESULTS- cont.

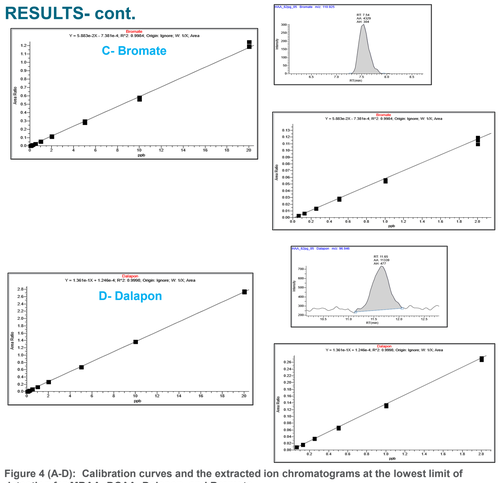


Figure 4 (A-D): Calibration curves and the extracted ion chromatograms at the lowest limit of detection for MBAA, DCAA, Dalapon, and Bromate

CONCLUSIONS

- All the analytes in this assay were detected at the lowest calibration level, and the accuracy was within the U.S. EPA specified criteria.
- The resolution between the matrix peaks and HAAs was excellent, which allowed for minimum interference in detection, as well as ensuring a cleaner ion source of the MS.
- The Dionex ICS-6000 ion chromatography system and the TSQ Fortis Plus mass spectrometer platform 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. (accessed August 2021) https://assets.thermofisher.com/TFS-Assets/CMD/posters/PN-64430-IC-MS-Haloacetic-Acids-Bromate-Dalapon-Water-EPA-557-ASMS2015-PN64430-EN.pdfNational Food Safety Standard on Maximum Residue Limits for Veterinary Drugs in Foods (GB 31650-2019). https://food.chemlinked.com/database/view/1661

2. Wang, X. et al. Disinfection byproducts in drinking water and regulatory compliance: A critical review. Front. Environ. Sci. Eng. 2015, 9, 3–15. https://doi.org/10.1007/s11783-014-0734-1

3. Gilchrist, E.S. A review of oxyhalide disinfection by-products determination in water by ion chromatography and ion chromatography-mass spectrometry Anal. Chim. Acta 2016 Oct 26; 942, 12–22. doi: 10.1016/j.aca.2016.09.006.

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