

## Application News

SSI-LCMS-134

Liquid Chromatography Mass Spectrometry

# Simultaneous LC-MS/MS Analysis of Haloacetic Acids, Bromate, Chlorate, and Dalapon in Water Samples



Liquid Chromatograph Mass Spectrometer  
**LCMS-8060NX**

### ■ Summary

During the disinfection of drinking water, disinfection byproducts are created. These include haloacetic acids, bromate, and chlorate. Haloacetic acids and bromate can cause cancer due to long-term exposure. The EPA has established Maximum Contaminant Levels for several of these compounds in water and they are regularly analyzed by water utilities and environmental laboratories. In this application note, a direct injection method was developed for the analysis of haloacetic acids, bromate, chlorate, and Dalapon in drinking water on a triple quad LCMS-8060NX using reverse phase chromatography. All compounds met a Limit of Quantitation (LOQ) of 1 µg/L or lower with a shorter run time than that of EPA 557 and minimal sample preparation.

### ■ Background

Chlorination is one of the most common processes to disinfect drinking water. When free chlorine reacts with naturally occurring organic matter, haloacetic acids are formed. These compounds have been associated with reproductive and developmental defects in laboratory animals.<sup>1</sup> The chlorination process also produces chlorate.<sup>3</sup> Bromate is formed in water due to bromide reacting with natural organic matter during disinfection with ozone, which has been known to cause kidney cancer and nervous system defects.<sup>2</sup> Dalapon is an herbicide used in agriculture. It is highly soluble in water, allowing it to end up in ground water and drinking water. Dalapon has been found to cause kidney effects.<sup>4</sup>

The U.S. Environment Protection Agency (EPA) currently regulates five haloacetic acids in drinking water according to the Stage 2 Disinfectants and Disinfection Byproducts Rule. The compounds—monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), dibromoacetic acid (DBAA), and trichloroacetic acid (TCAA)—are together known as HAA5. The maximum contaminant level (MCL) for the combined HAA5 is 60 µg/L.

The occurrence of four more HAAs (tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), chlorodibromoacetic acid (CDBAA), and bromodichloroacetic acid (BDCAA)) is being assessed under Unregulated Contaminant Rule 4 (UCMR4). HAA5 are regularly monitored by water utilities to comply with federal regulations. Requirements for the analysis of HAA9 beyond the scope of UCMR4 will be determined once the rule is completed; however, some utilities already monitor them as means of process control during water treatment. Bromate and dalapon are regulated by the EPA at an MCL of 10 µg/L and 200 mg/L, respectively.<sup>2</sup> Structures of all compounds are shown in Figure 1.

There are two EPA approved methods for the analysis of HAAs: EPA 552 and 557. EPA method 557 was developed as an alternative to EPA 552 and uses ion chromatography. Despite the minimal sample preparation required in EPA 557, the analysis time is very long. With so many compounds in one analysis, it is challenging to find a stationary phase that separates the small charged particles. This application note uses reverse phase chromatography and a shorter method time than EPA 557 to analyze HAA9, bromate, chlorate, and Dalapon in one injection and demonstrates that a LCMS-based method meets performance established in EPA 557.

### ■ Method

Haloacetic acids certified reference material EPA 522.2, potassium bromate, potassium chlorate, Dalapon, ammonium bicarbonate, ammonium chloride, potassium chloride, potassium sulfate, potassium nitrate were purchased from Sigma Aldrich (St. Louis, MO). Chloroacetic acid-2-<sup>13</sup>C, monobromoacetic acid-1-<sup>13</sup>C, dichloroacetic acid-2-<sup>13</sup>C, and trichloroacetic acid-2-<sup>13</sup>C were purchased from Dionex (Sunnyvale, CA). Formic acid was purchased from Jade Scientific (Westland, MI). LCMS grade methanol and water were purchased from Honeywell (Charlotte, NC).

A Shimadzu LC-30 Nexera HPLC system was coupled to an LCMS-8060NX. Electrospray ionization (ESI) in negative mode was used for ionization. The LC and MS parameters are shown in Tables 1 and 2, respectively. Table 3 lists the MRM transitions for all analytes.

A mixed stock solution of HAA9, bromate, chlorate, and Dalapon was created in LCMS grade water at 100 µg/mL. This was used to prepare a calibration curve from 0.05-100 µg/L in LCMS grade water. A laboratory fortified blank (LFB) was created by adding 100 mg/L ammonium chloride to LCMS grade water. A synthetic sample matrix (SSM) was created by adding the following to LCMS grade water: 100 mg/L ammonium chloride, 20 mg/L nitrate, 150 mg/L bicarbonate, 250 mg/L chloride, and 250 mg/L sulfate. Both the LFB and SSM were fortified with different concentrations of analytes. Six water samples were acquired from various locations near the laboratory. According to U.S. EPA Method 557, residual chlorine in samples was quenched with 100 mg/L ammonium chloride.<sup>5</sup> All standards and samples were spiked with internal standards at the following concentrations: chloroacetic acid-2-<sup>13</sup>C at 20 µg/L, monobromoacetic acid-1-<sup>13</sup>C at 10 µg/L, dichloroacetic acid-2-<sup>13</sup>C at 5 µg/L, and trichloroacetic acid-2-<sup>13</sup>C at 10 µg/L. Standards and samples were stored at 4°C during storage and acquisition.

A linear calibration curve of standards in LCMS grade water was injected three times. Samples were injected three times and quantified based on the slope of the calibration curves. LFB was injected six times to calculate recovery.

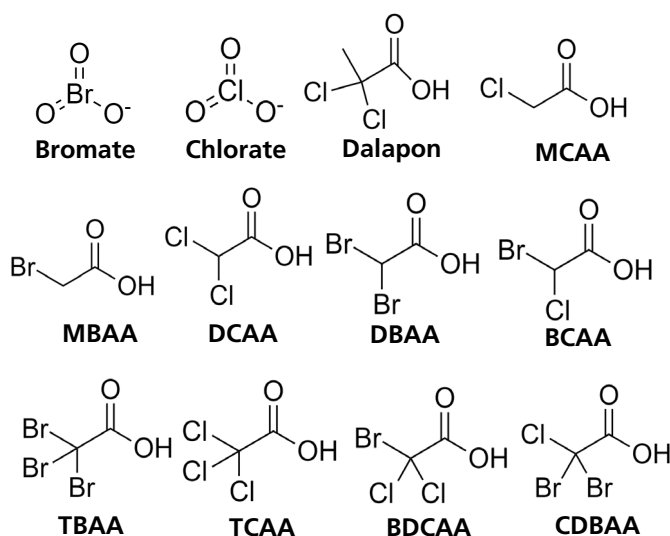


Figure 1: Structure of analytes

Table 1: Liquid Chromatography parameters

LC Conditions		
Mobile Phase A	0.05% formic acid in H <sub>2</sub> O	
Mobile Phase B	0.3% formic acid in 80:20 MeOH:H <sub>2</sub> O	
Flow Rate	0.5 mL/min	
Gradient	Time (min)	% B
	0-2	1
	6	40
	7	60
	12	100
	16	100
	16.1	1
	20	Stop
Column	Capcell Pak C18 MGIII 150x3mm, 3 µm	
Column Oven Temperature	25 °C	
Injection Volume	30 µL	

Table 2: Mass Spectrometry parameters

MS Conditions		
Nebulizing Gas	2 L/min	
Heating Gas Flow	Time (min)	Flow (L/min)
	0-10	20
	10-15	5
	15-20	20
Drying Gas Flow	20	
Interface Temperature	100 °C	
Desolvation Temperature	Time (min)	Temp (°C)
	0-10	130
	10-15	100
	15-20	130
Heat Block Temperature	75 °C	
Interface Voltage	1 kV	
Focus Voltage	2 kV	

**Table 3:** Optimized MRM acquisition parameters, retention times, and internal standard used for all analytes. Quantifier ions are in bold.

Compound	Internal Standard	RT (min)	Q1 (m/z)	Q3 (m/z)	CE (V)
BrO <sub>3</sub> <sup>-</sup>	MBAA-1- <sup>13</sup> C	2.707	<b>127.05</b> 127.05	<b>111.00</b> 95.00	<b>24.0</b> 30.0
ClO <sub>3</sub> <sup>-</sup>	MCAA-2- <sup>13</sup> C	3.244	<b>83.10</b> 83.10	<b>67.00</b> 51.05	<b>23.0</b> 29.0
MCAA	MCAA-2- <sup>13</sup> C	4.917	<b>93.05</b> 139.15	<b>35.05</b> 35.10	<b>13.0</b> 20.0
DCAA	DCAA-2- <sup>13</sup> C	6.819	<b>127.05</b> 173.10	<b>83.10</b> 83.05	<b>11.0</b> 16.0
MBAA	MBAA-1- <sup>13</sup> C	6.642	<b>183.00</b> 137.05	<b>79.00</b> 79.00	<b>22.0</b> 22.0
BCAA	DCAA-2- <sup>13</sup> C	8.200	<b>219.05</b> 173.05	<b>129.00</b> 81.05	<b>11.0</b> 22.0
DBAA	DCAA-2- <sup>13</sup> C	9.171	<b>262.95</b> 217.00	<b>172.80</b> 172.95	<b>17.0</b> 11.0
Dalapon	DCAA-2- <sup>13</sup> C	10.951	<b>141.05</b> 141.05	<b>97.00</b> 35.10	<b>11.0</b> 16.0
TCAA	TCAA-2- <sup>13</sup> C	11.572	<b>207.05</b> 163.05	<b>117.30</b> 119.00	<b>11.0</b> 10.0
BDCAA	TCAA-2- <sup>13</sup> C	12.032	<b>252.80</b> 252.80	<b>163.00</b> 81.00	<b>10.0</b> 23.0
CDBAA	TCAA-2- <sup>13</sup> C	12.391	<b>296.80</b> 207.00	<b>206.8</b> 79.2	<b>11.0</b> 13.0
TBAA	TCAA-2- <sup>13</sup> C	12.723	<b>340.85</b> 251.00	<b>250.85</b> 79.00	<b>12.0</b> 34.0
MCAA-2- <sup>13</sup> C		4.912	<b>94.05</b>	<b>35.10</b>	<b>16.0</b>
MBAA-1- <sup>13</sup> C		6.671	<b>138.05</b>	<b>78.90</b>	<b>22.0</b>
DCAA-2- <sup>13</sup> C		6.848	<b>128.05</b>	<b>84.00</b>	<b>11.0</b>
TCAA-2- <sup>13</sup> C		11.583	<b>162.05</b>	<b>118.05</b>	<b>9.0</b>

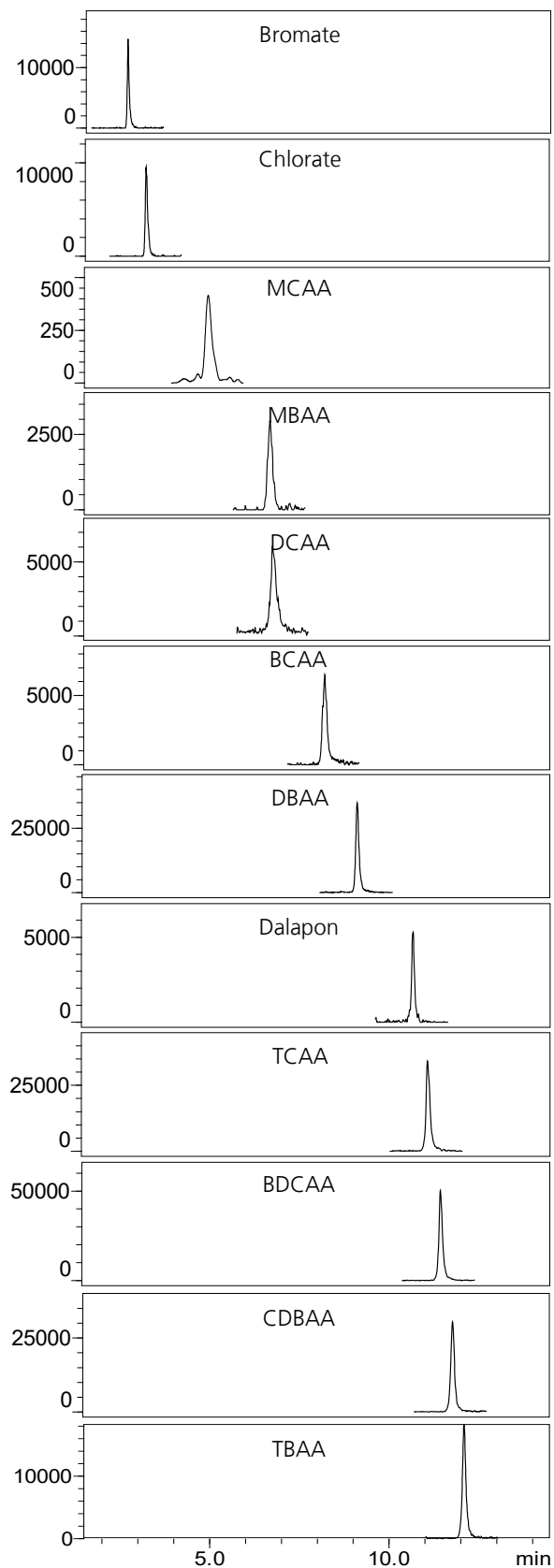
### ■ Results and Discussion

The mobile phase composition, gradient, column, column oven temperature, and interface parameters were optimized to achieve the best sensitivity and peak shape. The method developed includes bromate, chlorate, and dalapon in addition to the nine haloacetic acids.

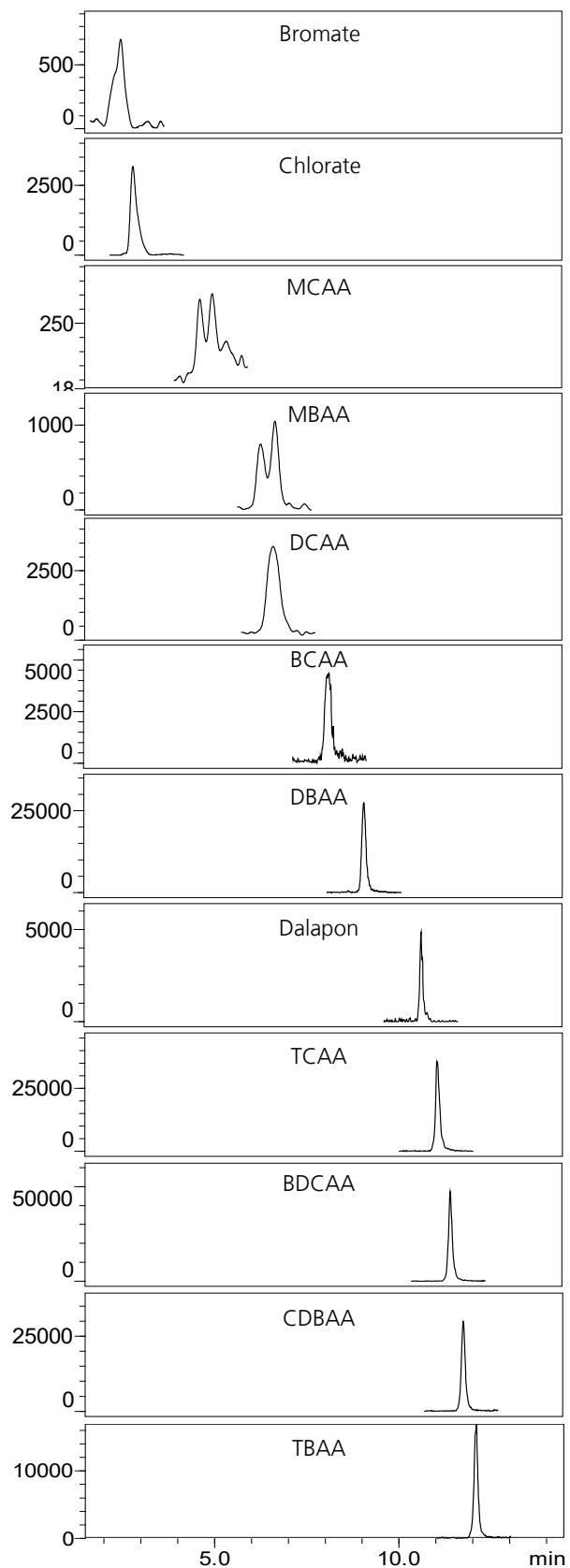
All compounds were quantified by an internal standard with a 20-minute separation method. Because many compounds have similar MRM transitions, reference ions were used as confirmation.

Figures 2 and 3 show chromatograms of all analytes in LCMS grade water and SSM, respectively. Peak shape was slightly affected by SSM.

The matrix was purposely made to contain a higher ionic concentration than typical drinking water.



**Figure 2:** Chromatogram of HAAs, bromate, chlorate, and Dalapon in LCMS grade water at 5 µg/L



**Figure 3:** Chromatogram of HAAs, bromate, chlorate, and Dalapon in synthetic sample matrix at 5 µg/L

Calibration curves were acquired in LCMS grade water in triplicate. Linear curves with a weighting of  $1/C^2$  were acquired with a coefficient of determination higher than 0.99 for all compounds. Accuracies for calibration points are within 80-120%. Figure 4 shows the calibration curves for the analytes. Table 4 shows the linear range, equation, and  $R^2$ .

Table 5 shows recoveries for all analytes in LFB. LCMS grade water was quenched with 100 mg/L ammonium chloride and fortified with analytes at 5  $\mu\text{g/L}$  and 100  $\mu\text{g/L}$ . Each LFB was injected six times to give recoveries ranging from 80-120% with percent RSD (relative standard deviation) less than 15%.

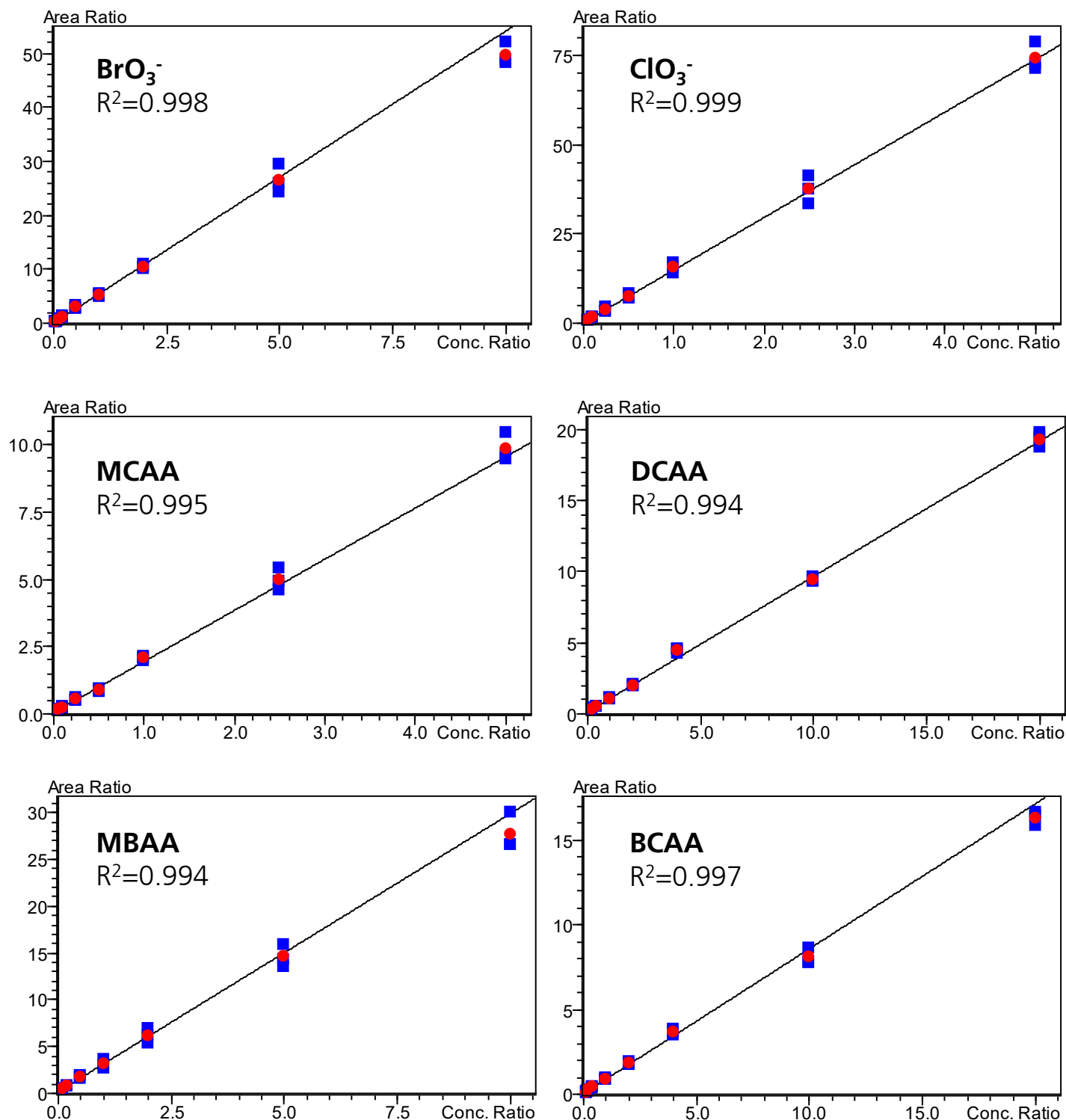
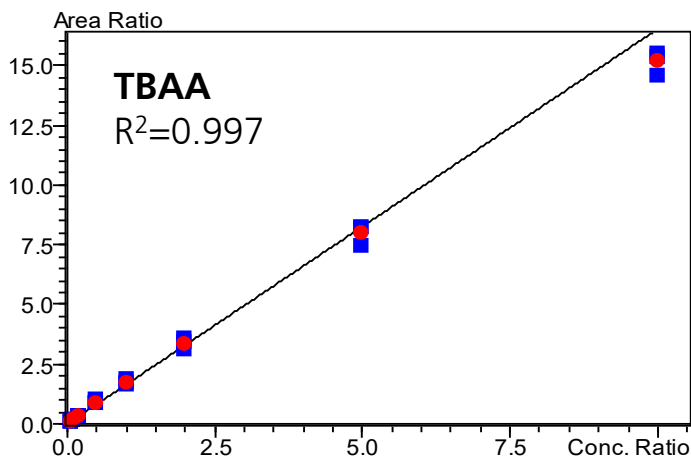
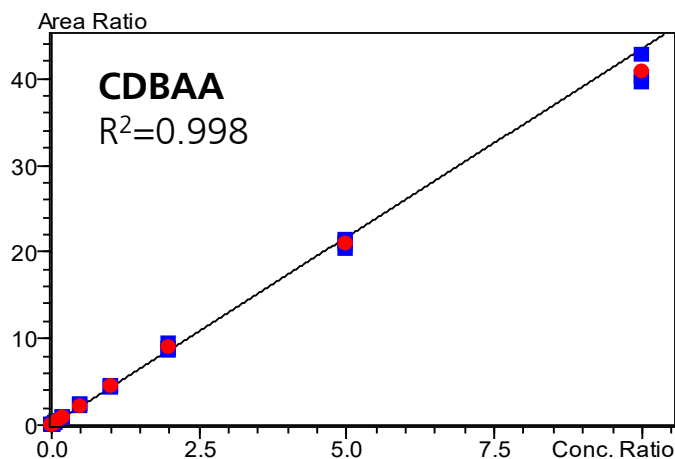
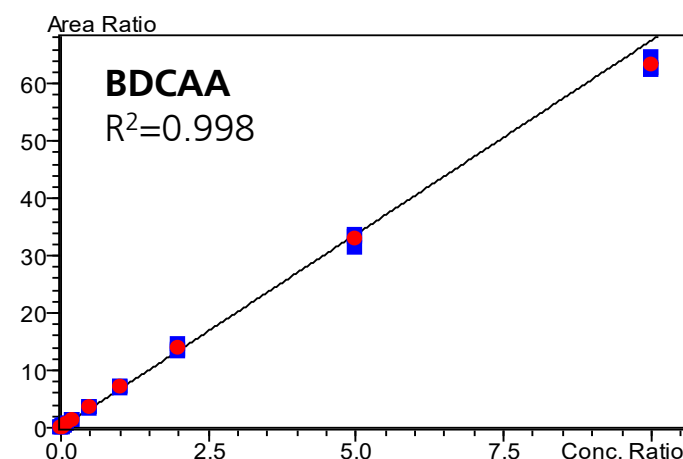
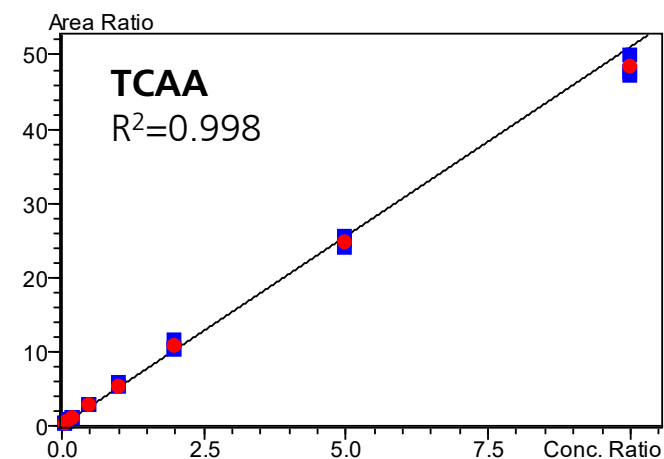
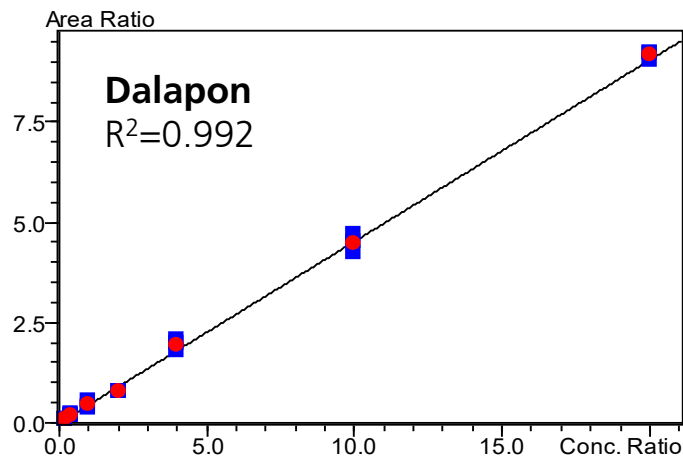
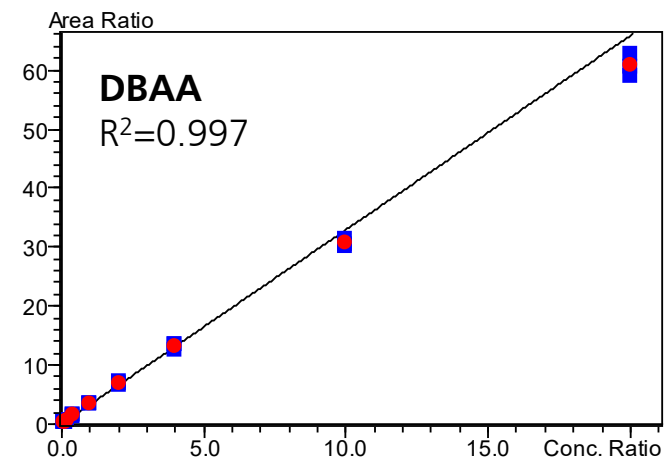


Figure 4: Calibration curves with  $R^2$  for all analytes in LCMS grade water; All curves were acquired in triplicate with a weighting of  $1/C^2$



**Figure 4 (Cont.):** Calibration curves with  $R^2$  for all analytes in LCMS grade water; All curves were acquired in triplicate with a weighting of  $1/C^2$ .

**Table 4:** Linear range, equation, and R<sup>2</sup> for all analytes in LCMS grade water.

Compound	Linear Range (µg/L)	Equation	R <sup>2</sup>
BrO <sub>3</sub> <sup>-</sup>	0.2 - 100	y = 5.40909x + 0.0531617	0.996
ClO <sub>3</sub> <sup>-</sup>	1 - 100	y = 14.7429x + 0.181382	0.999
MCAA	1 - 100	y = 1.90020x + 0.0478739	0.995
DCAA	1 - 100	y = 0.951358x + 0.155216	0.994
MBAA	1 - 100	y = 2.97190x + 0.179669	0.994
BCAA	0.5 - 100	y = 0.856335x + 0.0405047	0.997
DBAA	0.2 - 100	y = 3.29039x + 0.0582928	0.997
Dalapon	1 - 100	y = 0.452201x - 0.00154893	0.992
TCAA	0.5 - 100	y = 5.10336x + 0.0835828	0.998
BDCAA	0.1 - 100	y = 6.75272x + 0.0135085	0.998
CDBAA	0.1 - 100	y = 4.34436x + 0.00850562	0.998
TBAA	0.5 - 100	y = 1.65312x - 1.77153e-005	0.998

**Table 5:** Recoveries of analytes in Laboratory Fortified Blanks at 5 and 100 µg/L.

Compound	Linear Range (µg/L)	Recovery (%) (mean ± %RSD, n=6)	
		5 µg/L	100 µg/L
BrO <sub>3</sub> <sup>-</sup>	0.2 - 100	98 ± 10	86 ± 6
ClO <sub>3</sub> <sup>-</sup>	1 - 100	103 ± 13	90 ± 7
MCAA	1 - 100	101 ± 14	90 ± 6
DCAA	1 - 100	100 ± 4	89 ± 4
MBAA	1 - 100	93 ± 7	85 ± 7
BCAA	0.5 - 100	106 ± 9	89 ± 5
DBAA	0.2 - 100	109 ± 3	88 ± 6
Dalapon	1 - 100	117 ± 6	99 ± 9
TCAA	0.5 - 100	107 ± 3	91 ± 5
BDCAA	0.1 - 100	102 ± 4	104 ± 4
CDBAA	0.1 - 100	103 ± 3	101 ± 4
TBAA	0.5 - 100	105 ± 9	85 ± 9

**Table 6:** Concentrations of HAAs, bromate, chlorate, and Dalapon in water samples  
(mean  $\pm$  standard deviation ( $\mu\text{g/L}$ ), n=3)

Compound	DI Water	Tap Water A	Tap Water B	Tap Water C	River	Stream
$\text{BrO}_3^-$	ND	ND	ND	ND	ND	ND
$\text{ClO}_3^-$	ND	250 $\pm$ 20	220 $\pm$ 30	400 $\pm$ 50	16.1 $\pm$ 0.3	ND
MCAA	ND	ND	ND	ND	ND	ND
DCAA	ND	9.8 $\pm$ 0.7	ND	ND	ND	ND
MBAA	ND	ND	ND	ND	ND	ND
BCAA	ND	1.2 $\pm$ 0.1	ND	ND	ND	ND
DBAA	ND	ND	ND	ND	ND	ND
Dalapon	ND	1.5 $\pm$ 0.2	3.2 $\pm$ 0.8	ND	ND	ND
TCAA	ND	19 $\pm$ 1.0	15.5 $\pm$ 0.3	<LOQ	<LOQ	<LOQ
BDCAA	ND	3.57 $\pm$ 0.11	1.639 $\pm$ 0.010	0.16 $\pm$ 0.04	ND	ND
CDBAA	<LOQ	0.38 $\pm$ 0.09	<LOQ	<LOQ	ND	ND
TBAA	ND	ND	ND	ND	ND	ND
Total of HAAs	0	34.3	17.2	0.2	0	0

Six water samples were acquired from locations near the laboratory. Each sample was quenched with 100 mg/L ammonium chloride and spiked with internal standards. The samples were injected three times. The matrix effects of sensitivity and peak shape that were seen in the SSM are not present in the water samples. TCAA is the most abundant of all the HAAs in the samples. None of the samples are over the MCL of 60  $\mu\text{g/L}$  for HAA5, or over the MCL of bromate at 10  $\mu\text{g/L}$  and Dalapon at 200 mg/L.

#### ■ Conclusion

A method was developed to analyze nine haloacetic acids, bromate, chlorate, and dalapon in a 20-minute injection. This method is shorter than the one used by EPA and uses reverse phase chromatography coupled to the Shimadzu triple quadrupole LCMS-8060NX. The instrument demonstrates excellent linearity and accuracy for all analytes, giving calibration curves with  $R^2$  greater than 0.99. Recoveries for the method were between 80 and 120% with RSD less than 15%.

The method experienced minimal matrix effects when applied to a synthetic sample matrix and water samples. Analytes were successfully quantified in water samples without sensitivity or peak shape loss. This method developed on the LCMS-8060NX proved to be sensitive and selective to quantify 12 carcinogenic compounds in water.

#### ■ References

1. Haloacetic Acids <https://drinktap.org/Water-Info/Whats-in-My-Water/Haloacetic-Acids> (accessed 2021 -06 -17).
2. EPA | Envirofacts | ICR [https://archive.epa.gov/enviro/html/icr/web/html/gloss\\_dbp.html#hha](https://archive.epa.gov/enviro/html/icr/web/html/gloss_dbp.html#hha) (accessed 2021 -06 -17).
3. WHO. Chlorite and Chlorate in Drinking-Water. 2005.
4. EPA. Technical Factsheet on: DALAPON. In *National Primary Drinking Water Regulations*.
5. 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). September 2009.



# UAFMS

ULTRA FAST MASS SPECTROMETRY



LCMS-8040



LCMS-8045



LCMS-8050



LCMS-8060NX



LCMS-2020



Q-TOF LCMS-9030

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First Edition: July 2021