Application News

No. HPLC-044

High Performance Liquid Chromatography

Determination of Oxyhalides in Water Using Suppressed Anion Chromatography Coupled with Single Quadrupole MS

■ **Introduction**

In order to protect public health, drinking water is usually disinfected using chlorine, chlorine dioxide, sodium hypochlorite and ozone. However, disinfection will not only remove microorganisms that cause diseases in humans, but also produce disinfection byproducts (DBPs), including chlorite, chlorate and bromate. It is reported that a significant amount of chlorite and chlorate can be formed during water chlorination and bromate can be produced from water ozonation when water contains high levels of bromide. These DBPs are potentially harmful to humans. For example, bromate has been identified as a potential human carcinogen. Therefore, the Environmental Protection Agency (EPA) of the United States has proposed that the maximum concentration level (MCL) of bromate is 10ppb.

Ion Chromatography (IC) is a widely used technique for the analysis of inorganic anions in environmental samples. IC with suppressed conductivity detection has been a regulated method for the determination of inorganic anions in water, including DBPs, for two decades. The method is described in EPA method 300.1[1]. The performance of the Shimadzu Prominence IC for meeting requirements outlined in EPA method 300.1 was published in the Application Note HPLC-022[2]. The detection limits achieved with the suppressed IC method were in the low ppb range. However, reaching these levels may be challenging during the analysis of complex environmental samples. Alternative methods, such as post-column derivatization or 2D chromatography (i.e. EPA method 317,326,302…), may be needed to achieve the desired performance. Nowadays, with the growing of routine use of mass spectrometry (MS), the advantage of IC coupled with MS has been investigated to improve the sensitivity and selectivity for accurate measurement of ionic substances at trace levels of concentration in a complex sample matrix.

In this study, an IC-MS method was developed using a novel electrolytically regenerated suppressor as a part of a modular IC system coupled with a LCMS-2020 single quadrupole MS for the determination of oxyhalide anions, including chlorite, chlorate and bromate, in drinking water. Both conductivity detection (CDD) and MS with electrospray ionization in negative mode were used in the method. The CDD is used to monitor the column effluent; only the oxyhalides of interest are directed to MS via a divert valve. Quantitative analysis was performed by using the selected ion monitoring (SIM) on two isotopic mass of each compound. The system produced a linear response with a correlation coefficient greater than 0.999 from a concentration range of 0.5-100 ppb for chlorite and chlorate, and 0.5 -25 ppb for bromate. This study demonstrated robust and reliable performance of the novel modular IC-MS for the determination of oxyhalides in drinking water.

■ **Experimental**

Equipment
Experiments were performed using a modular Shimadzu IC system coupled with a single quadrupole MS, which consists of the following components:

- System controller CBM-40 lite
- Shimadzu degasser DGU-403
- Shimadzu LC-20Ai pump
- Shimadzu autosampler SIL-20AC with inert kit
- Shimadzu column oven CTO-40S
- Shimadzu conductivity detector CDD-10Avp
- Shimadzu electrolytic suppressor ICDS-40A
- Shimadzu single quadrupole MS LCMS-2020
- Shimadzu LabSolutions chromatography data system software version 5.97 was used for all experiments

Chemicals and Materials
Reagents and Standards
- Degassed deionized water with resistivity equal or greater than 18.0 MΩ-cm
- Sodium carbonate (reagent grade or better) was obtained from Sigma-Aldrich
- Bromate standard, 1000 ppm in H_2O, 100 mL (Shimadzu P/N: 220-91638-67)
- Chlorate standard, 1000 ppm in H_2O, 100 mL (Shimadzu P/N: 220-91638-68)
- Chlorite standard, 1000 ppm in H_2O, 100 mL (Shimadzu P/N: 220-91638-69)

Eluent and standards preparation
Preparation of 1 L of 0.18 M stock sodium carbonate; dissolve 19.08 g sodium carbonate (Na_2CO_3) in deionized water and dilute to 1 L. Preparation of 1 L of eluent (5.4 mM sodium carbonate); pipet 30 mL of stock sodium carbonate then dilute to the mark of a volumetric flask (1 L) with deionized water.

Standards at different concentrations were prepared by diluting from the stock standards using degassed deionized water with resistivity equal to or greater than 18.0 MΩ-cm. Bottled water was purchased from local merchants; tap water, well water and dispenser water samples were collected in 50 mL plastic containers. All samples were stored at 4 °C, protected from light and used in less than seven days. Polypropylene sample vials and containers were used in the study. Samples were filtered through 0.45 µm polyethersulfone (PES) filters.

IC-MS conditions
The method conditions for both IC and MS are shown in Table 1.

Table 1: Method Conditions

IC (Prominence)	
Column	Shodex IC SI-52 E, 4 x 250 mm (P/N 220-91524-04)
	Guard column, Shodex SI-90G, 4.6 x 10 mm (P/N 220-91524-05)
Eluent	5.4 mM sodium carbonate
Flow rate	0.5 mL/min
Column oven	50 °C
Injection volume	300 µL
Detection	Suppressed conductivity;
	180 mA in external water mode, water flow rate is 1.2 mL/min
Run time	21 min
MS (LCMS-2020)	
Ionization	ESI -
Interface temperature	350 °C
DL temperature	200 °C
Nebulizing gas flow	1.5 L/min
Heat block	500 °C
Drying gas flow	15 L/min
Interface voltage	-5 kv
DL voltage	-20 v
Scan range (m/z)	20-200
Selected ions m/z	66.9, 68.9, 82.9, 84.9, 126.8, 128.8

■ **Results and Discussion**
Ion Chromatography (IC) is a widely used technique for the analysis of inorganic anions, cations and low-molecular-weight organic ionic substances. The separation of IC is normally performed with columns containing low capacity, ion-exchange stationary phase. In this study, a polymer-based anion-exchange column containing quaternary ammonium group was used to accomplish the separation. Sodium ions of the carbonate buffers and any analyte counter cations from eluent were removed by an electrolytically regenerated suppressor prior to entering the conductivity detector flow cell and mass spectrometer. The schematic diagram of the IC-MS system used in this study is illustrated in Figure 1.

In this method, external fresh D.I water is continuously pumped through the suppressor regeneration channel at a 1.2 mL/min flow rate using the second pump, and hydronium ions are continuously generated from electrodialysis and exchanged with sodium ions, which convert salts to their corresponding acids. The conductivity detector is used to monitor column effluent. Only the oxyhalides of interest are directed to the MS using a divert valve. The desalting of the suppressor not only reduces the conductivity of the eluent, allowing high-sensitivity analysis of anions during conductivity detection, but also protects the MS from a buildup of trace salts from the eluent post-suppressor.
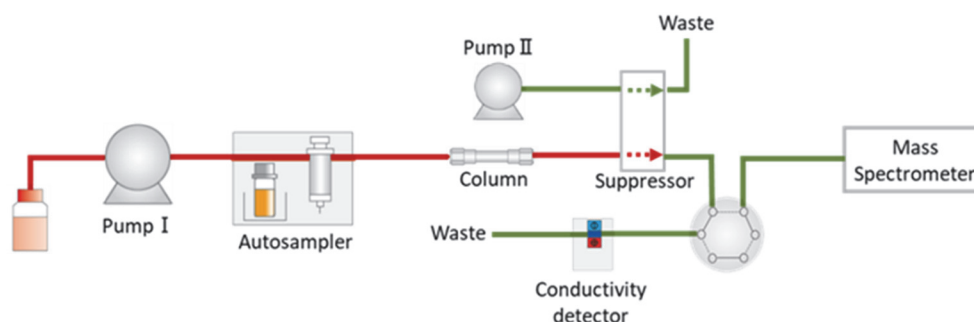
Figure 1: Schematic diagram of IC-MS system for oxyhalides analysis.

Both qualitative and quantitative methods for the analysis of oxyhalide anions, including chlorite, chlorate and bromate in drinking water, were developed in the study. Qualitative analysis was performed by scanning samples from *m/z* 20 to 200 in ESI negative mode. Quantitative analysis was performed by using the selective ion monitoring (SIM) mode to monitor chlorite, bromate and chlorate ions at their specific mass to charge ratio (m/z). With the fast scan speed of the instrument, both scan and SIM data can be obtained in one run.

Qualitative analysis was done by obtaining the retention time and mass spectrum of analytes. The method details of the chromatography and mass spectrometry are listed in Table 1. Figure 2 shows that chlorite and chlorate eluted at 10.5 min and 18.8 min, respectively. The 3:1 isotopic mass to charge ratio (m/z) of 66.9 to 68.9 and 82.9 to 84.9 were observed for chlorite and chlorate, respectively. Bromate was eluted at 10.9 min, and around 1:1 isotopic ratio of m/z 126.8 to 128.8 was detected.
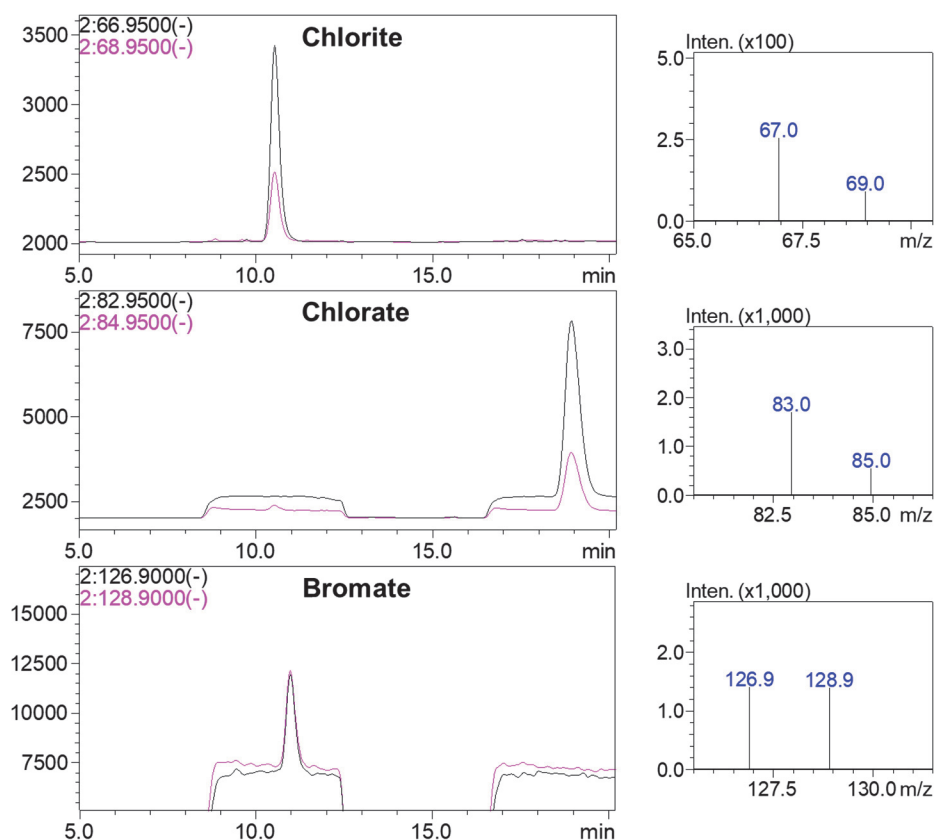
Figure 2: Ion chromatogram and corresponding mass spectrometer (SIM mode) of mixed chlorite, chlorate and bromate standard (5 ppb each).

Linearity and Method Detection Limit

Six-point calibration curves were established across the concentration range of 0.5-100 ppb for chlorite and chlorate, and 0.5 -25 ppb for bromate. Triplicate results were obtained and weighing of 1/c was applied on the calibration curve.

Correlation coefficients of $r^2 > 0.999$ with good accuracy (between 82% and 120%) were obtained for all three DBPs at each isotopic mass as shown in Figure 3.
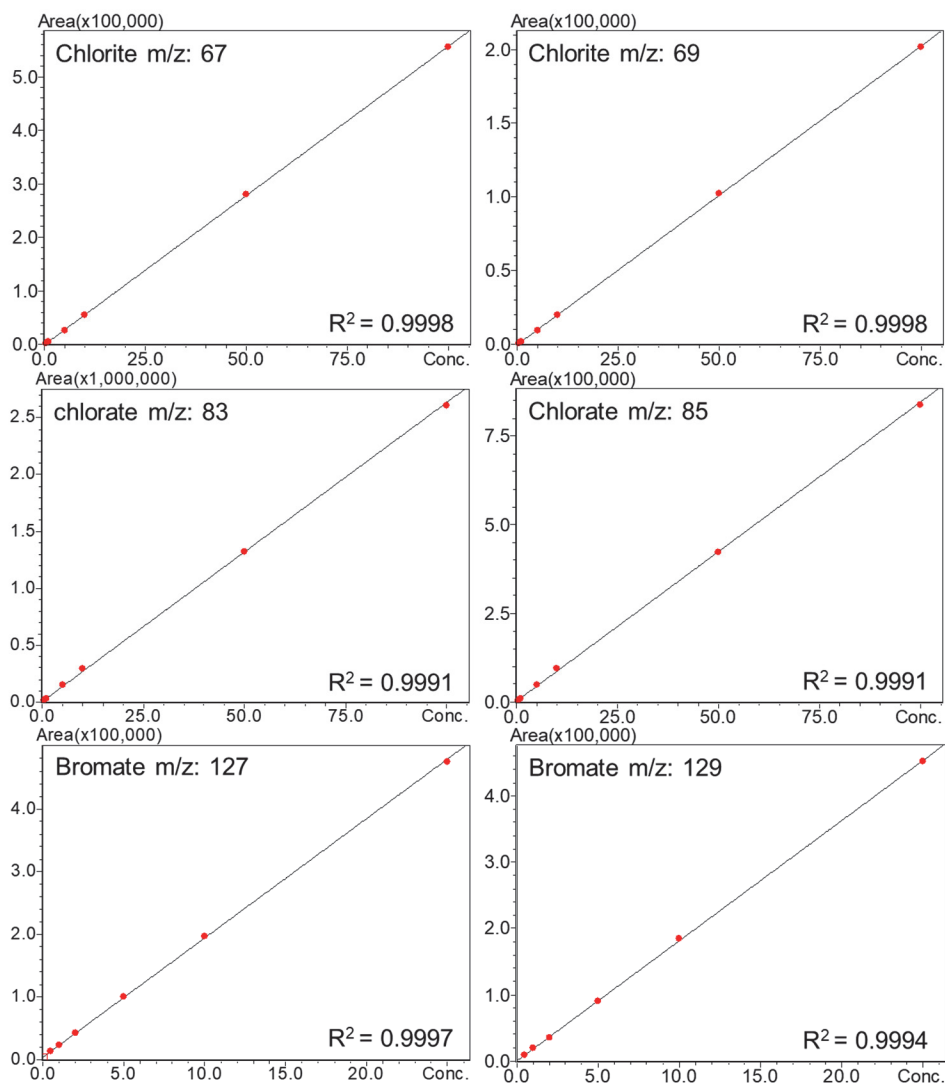

Figure 3: Calibration curves of chlorite, chlorate and bromate.

The Method Detection Limit (MDL) is a statistically calculated concentration, defined as the minimum concentration of a compound that can be measured with 99% confidence that the measured concentration is distinguishable from a blank. The MDL was investigated by making seven replicate injections of a mixed standard with a concentration of 0.5 ppb for each compound.

The MDL was calculated as (t) x (S), where t is student's t value for 99% confidence level (t = 3.14 for seven replicates) and S is standard deviation from seven injections. The MDL results for each compound are shown in Table 2. The linearity and MDL results show that good linearity and sensitivity are obtained from both isotopic mass for each compound. The quantification can be performed using either isotopic mass.

Table 2: MDL standards and MDL of chlorite, chlorate and bromate

Compounds	m/z	Calibration range (ppb)	Linearity	MDL Standard (ppb)	Calculated MDL (ppb)
Chlorite ($^{35}ClO_2^-$)	66.9	0.5-100	0.9998	0.5	0.08
Chlorite ($^{37}ClO_2^-$)	68.9	0.5-100	0.9998	0.5	0.08
Chlorate ($^{35}ClO_3^-$)	82.9	0.5-100	0.9991	0.5	0.06
Chlorate ($^{37}ClO_3^-$)	84.9	0.5-100	0.9991	0.5	0.07
Bromate ($^{79}BrO_3^-$)	126.8	0.5-25	0.9997	0.5	0.10
Bromate ($^{81}BrO_3^-$)	128.8	0.5-25	0.9994	0.5	0.07

Method precision

Method precision was performed using three mixed standards with concentrations of 0.5 ppb, 1ppb and 10 ppb, respectively. Table 3 shows the percent relative standard deviation (RSD) of the concentration from seven replicate injections of the three mixed standards for each compound. As shown in the table, excellent reproducibility (less than 3% RSD) was achieved for the samples at concentrations of 1 ppb and 10 ppb. Although the RSD is slightly increased at the concentration of 0.5 ppb, it is still less than 6.5% for each compound.

Determination of oxyhalides in water samples

Six water samples. including bottled water, tap water, dispenser water and well water, were analyzed in this study. The results of the measurement from three replicates are shown in Table 4. The recovery and accuracy of the method were investigated by spiking a mixed standard with a concentration of 10 ppb into the six water samples. A good recovery range of 84.1%-113.6 for all three oxyhalides was obtained from 3 replicates as shown in Table 5

Table 3: Method precision from seven replicates

Compounds	m/z	Standard (ppb)	Conc. (% RSD)	Standard (ppb)	Conc. (% RSD)	Standard (ppb)	Conc. (% RSD)
Chlorite ($^{35}ClO_2^-$)	66.9	0.5	4.6	1	3.7	10	0.69
Chlorite ($^{37}ClO_2^-$)	68.9	0.5	4.0	1	3.2	10	0.95
Chlorate ($^{35}ClO_3^-$)	82.9	0.5	5.3	1	3.3	10	0.41
Chlorate ($^{37}ClO_3^-$)	84.9	0.5	5.4	1	3.7	10	0.44
Bromate ($^{79}BrO_3^-$)	126.8	0.5	6.5	1	3.8	10	0.74
Bromate ($^{81}BrO_3^-$)	128.8	0.5	4.4	1	2.8	10	2.78

Table 4: Water samples analysis

Compounds	m/z	Bottled water (ppb)	Tap water (ppb)	Dispenser Water (ppb)	Well water 1 (ppb)	Well water 2 (ppb)	Well water 3 (ppb)
Chlorite ($^{35}ClO_2^-$)	66.9	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Chlorite ($^{37}ClO_2^-$)	68.9	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Chlorate ($^{35}ClO_3^-$)	82.9	0.5	76.1	90.7	0.2	51.0	< 0.1
Chlorate ($^{37}ClO_3^-$)	84.9	0.5	76.1	91.0	0.2	51.6	< 0.1
Bromate ($^{79}BrO_3^-$)	126.8	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Bromate ($^{81}BrO_3^-$)	128.8	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Table 5: Recovery of chlorite, chlorate and bromate in different water samples

Compounds	m/z	Added concentration (ppb)	Bottle water recovery (%)	Tap water Recovery (%)	Dispenser water recovery (%)	Well water 1 Recovery (%)	Well water 2 Recovery (%)	Well water 3 Recovery (%)
Chlorite ($^{35}ClO_2^-$)	66.9	10	105.0	104.4	104.5	100.1	89.0	99.2
Chlorite ($^{37}ClO_2^-$)	68.9	10	106.6	105.8	105.1	99.8	91.6	100.0
Chlorate ($^{35}ClO_3^-$)	82.9	10	104.6	100.8	84.5	105.8	96.0	101.6
Chlorate ($^{37}ClO_3^-$)	84.9	10	104.0	98.0	84.1	105.3	89.4	102.2
Bromate ($^{79}BrO_3^-$)	126.8	10	106.8	113.6	107.5	107.2	99.7	110.7
Bromate ($^{81}BrO_3^-$)	128.8	10	97.4	98.5	96.1	97.2	85.0	97.0

■ Conclusion

A sensitive and simple quantitative IC-MS method for determination of three oxyhalides, including chlorite, chlorate and bromate, was developed using a Shimadzu Prominence IC coupled with a single quadruple MS (LCMS-2020). This method demonstrates the increased sensitivity and specificity of IC-MS for the determination of trace amounts of oxyhalide in water compared to IC with only suppressed conductivity detection (i.e. EPA 300 or 300.1). All three oxyhalide anions can be simultaneously quantified at less than 0.5 ppb without using any laborious sample preparation or post-column additions to increase sensitivity.

With mass identification, more specific and confident results can be obtained using this method, especially in complex matrices. Additionally, this method minimizes errors and increases productivity in the laboratory and can be an alternative to conventional methods for the analysis of oxyhalides in water.

■ References

1. EPA Method 300.1 Determination of inorganic anions in drinking water by ion chromatography Revision 1.0.
2. Shimadzu Application Note HPLC-022, The determination of 10 anions in EPA Method 300.1 using Shimadzu high-resolution ion chromatography.

First Edition: February 2021

SHIMADZU

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com