APPLICATION NOTE

HPLC/ICP-MS

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Advances in Bromine Speciation by HPLC/ICP-MS

Introduction

Bromine is a natural component found in waters, most commonly as the bromide ion, Br^-. A common procedure for purifying drinking waters is treatment with ozone to kill bacteria. A byproduct of ozonolysis is the conversion of bromide to bromate (BrO_3^-), a known carcinogen. Therefore, a need exists to measure both bromide and bromate in drinking waters, as opposed to total bromine content.

Our earlier work on bromine speciation focused on separating Br^- and BrO_3^- via anion exchange HPLC and detecting the species with ICP-MS.[1] The method proved rugged, but required eight minutes per sample. This work focuses on significantly decreasing the analysis time and also explores the possibility of separating other bromine-containing compounds which were found in several water samples.

Experimental

HPLC Conditions

A PerkinElmer® Series 200 HPLC system, consisting of a quaternary pump, autosampler (with polypropylene vials), vacuum degasser and peltier column oven, was used for all analyses. The separation was done with an anion exchange column (ZirChrom®-SAX ; ZirChrom Separations, Anoka, MN USA).

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Both isocratic and gradient HPLC methods were explored. For samples containing only bromide and bromate, the isocratic method was preferred due to the higher sample throughput. For samples containing additional bromine compounds, the gradient method was used. Details of both methods are shown in Tables 1 and 2, respectively. It should be noted that no pH adjustments were made on the mobile phases; the pHs used were those that resulted from mixing the mobile phase components at the concentrations specified.

Table 1. **HPLC Isocratic Method Parameters.**

HPLC System	PerkinElmer Series 200 Quaternary Pump, Autosampler, Vacuum Degasser, Peltier Column Oven
Column	ZirChrom®-SAX (3 µm, 100 x 4.6 mm)
Mobile Phase	18 mM NH_4OH + 3 mM HNO_3
pH	10.2
pH Adjustment	None
Flow Rate	1.5 mL/min
Column Temperature	50 °C
Injection Volume	50 µL
Run Time	4 minutes
Total Analysis Time	4 minutes

Table 2. **HPLC Gradient Method Parameters.**

HPLC System	PerkinElmer Series 200 Quaternary Pump, Autosampler, Vacuum Degasser, Peltier Column Oven
Column	ZirChrom®-SAX (3 µm, 100 x 4.6 mm)
Solvent A	14 mM NH_4OH + 6 mM HNO_3; pH=7.3
Solvent B	18 mM NH_4OH + 3 mM HNO_3; pH=10.2
Gradient Profile	2 min at 100% A Step to 100% B 4 min at 100% B
Re-equilibration Time	5 min
pH Adjustment	None
Flow Rate	1.5 mL/min
Column Temperature	50 °C
Injection Volume	50 µL
Run Time	6 min
Total Analysis Time	11 min

ICP-MS conditions

Detection of the HPLC eluent was accomplished with an ELAN® DRC II ICP-MS (PerkinElmer, Inc. Shelton, CT). Instrumental conditions are listed in Table 3. All analyses were done in standard mode (i.e. no reaction gas was used); monitoring Br^+ at m/z 79. Those samples which produced chromatograms containing additional peaks were also analyzed in DRC mode, monitoring BrO^+ at m/z 95 and 97. The presence of the same peaks at both bromine isotopes in DRC mode (as well as in standard mode) confirms that the additional peaks contain bromine and are not the result of interferences.

Standards and samples

Bromide and bromate standards were made daily from 1000 mg/L stock solutions (High Purity Standards, Charleston, SC) by dilution in 18 MΩ-cm water.

Samples consisted of bottled waters purchased at local grocery stores from various countries and water collected directly from the tap. No sample preparation or dilutions were used, aside from filtering the waters which contained visible particulates.

Software

All instrument control and data processing and analysis was accomplished with Chromera® software (PerkinElmer, Inc. Shelton, CT). Peak areas and external calibration curves were used for quantitative measurements. The calibration standards were made in 18 MΩ-cm water, and the levels were chosen to cover the range where the majority of species were found, although occasional samples produced concentrations higher than the highest calibration standard.

Table 3. **ICP-MS Conditions.**

Instrument	PerkinElmer ELAN DRC II
Nebulizer	Quartz Concentric
Spray Chamber	Quartz Cyclonic
RF Power	1500 W
Dwell Time	250 ms
Analytes	Standard Mode – $^{79}Br^+$ DRC Mode – $^{79,81}BrO^+$ (m/z 95, 97)
Reaction Gas	Standard Mode – None DRC Mode – N_2O = 0.5
RPq	Standard Mode – 0.25 DRC Mode – 0.50

Results and discussion

Figure 1 shows a chromatogram of a 10 µg/L mixed standard containing both bromate and bromide. The species are separated and baseline-resolved in less than three minutes. Figure 2 displays a chromatogram of a 1 µg/L mixed standard. The intensities of the peaks are about two times baseline noise, signifying the lower level which can be measured. Larger injection volumes would allow lower levels to be measured, but could overload the column at higher concentrations.

Once the separation was established, the method was applied to a number of drinking water samples. To determine the reliability of the method, the samples were measured in duplicate on four non-consecutive days; the results appear in Table 4. The small variation in the results indicates the robustness of the method. Additionally, a single sample was analyzed 49 times consecutively over a period of 3.75 hours. Figure 3 shows the chromatograms overlayed from this study, as well as the average concentration and standard deviation of the measurements. Additionally, the relative standard deviation of the retention times for both peaks is 0.5 and 0.4, respectively. Taken together, these tests demonstrate the ruggedness and repeatability of the method.

Figure 4 displays chromatograms of two samples which contain peaks in addition to bromate and bromide. To confirm that these peaks are really Br-containing species and not interferences, these samples were analyzed in DRC mode. For this study, BrO^+ was monitored at m/z 95 and 97, representing both bromine isotopes. The conversion of Br^+ to BrO^+ was accomplished by reaction with N_2O in the reaction cell, according to the following gas phase chemical reaction:[2]

$$Br^+ + N_2O \rightarrow BrO^+ + N_2$$

$$k = 2.80 \times 10^{-10}\ cm^3s^{-1}$$

The relatively high value of the rate constant k signifies that the reaction occurs rapidly, indicating that it is easily accomplished in the reaction cell.

Figure 5 shows overlayed chromatograms of BrO^+ at m/z 95 and 97 for the two samples displayed in Figure 4. Because the chromatograms of both bromine isotopes in DRC mode are the same and match the chromatograms acquired in standard mode (Br 79), it can be concluded that the extra peaks are Br-containing species and not interferences.

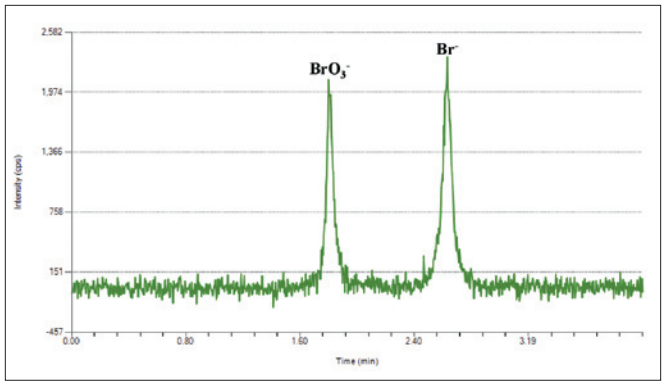

Figure 1. Chromatogram (isocratic LC method) of a standard containing 10 µg/L of bromide and bromate.

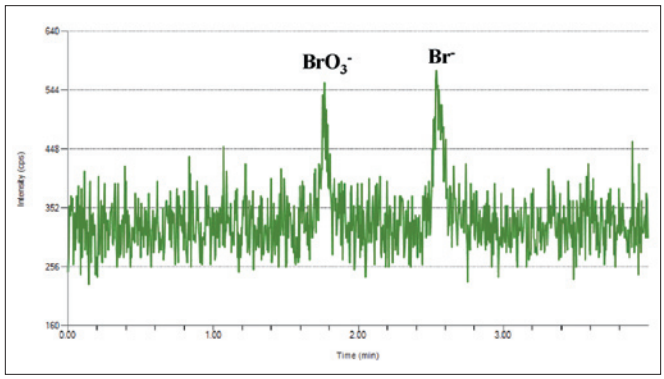

Figure 2. Chromatogram (isocratic LC method) of a standard containing 1 µg/L of bromide and bromate.

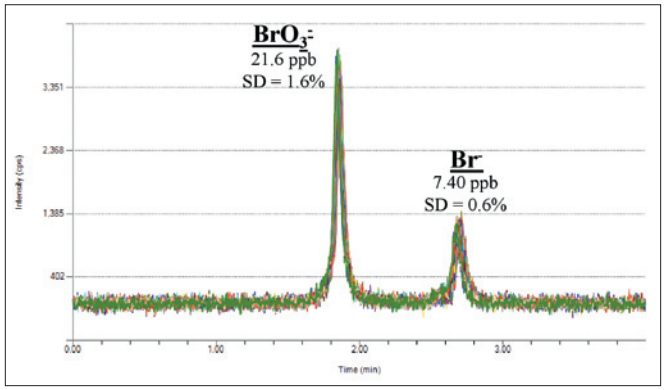

Figure 3. Overlay of 24 chromatograms (isocratic LC method) of a water sample obtained by consecutive injections. The average concentrations and standard deviation for each species are shown.

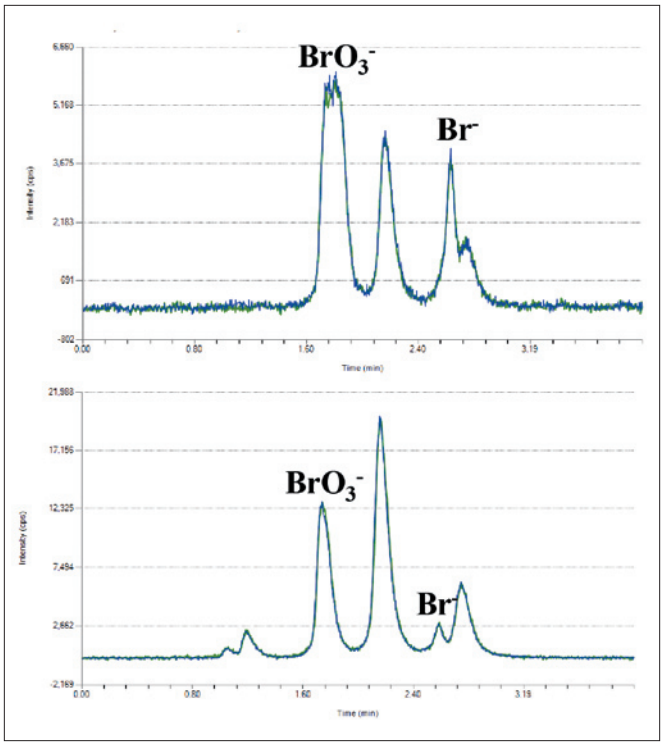

Figure 4. Chromatograms (isocratic LC method) of a water sample containing multiple peaks: bromide, bromate and unidentified species.

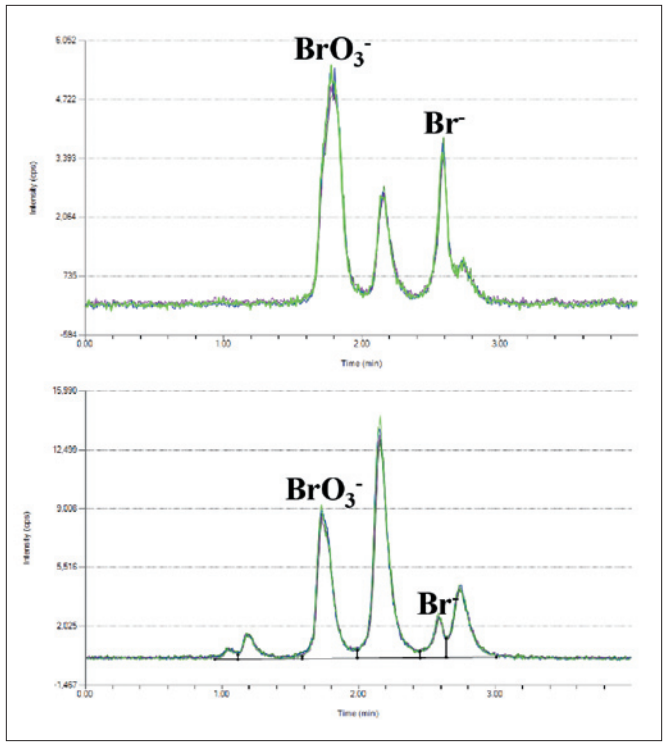

Figure 5. Chromatograms (isocratic LC method) of the same samples as in Figure 4, but acquired in DRC mode. For each sample, two chromatograms are overlayed: BrO^+ at m/z 95 and 97.

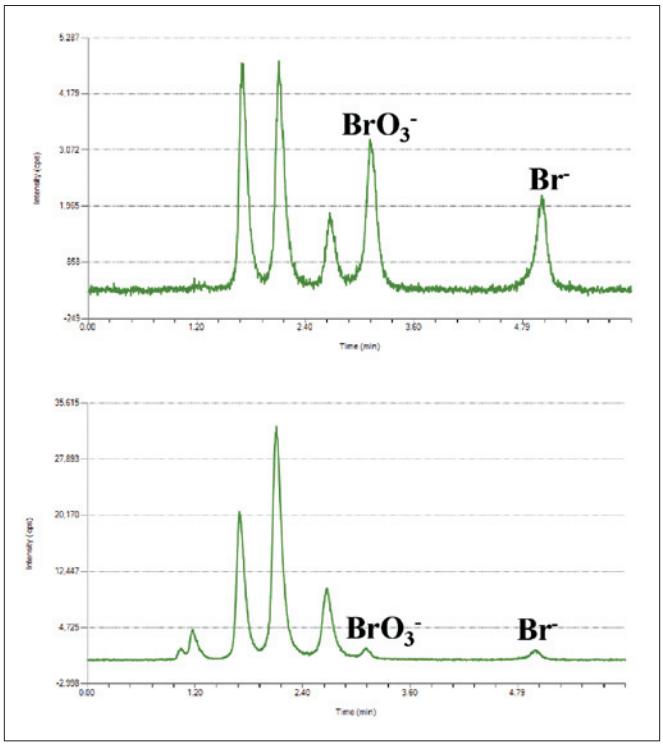

Figure 6. Chromatograms of the samples in Figure 4, but obtained with a gradient LC method.

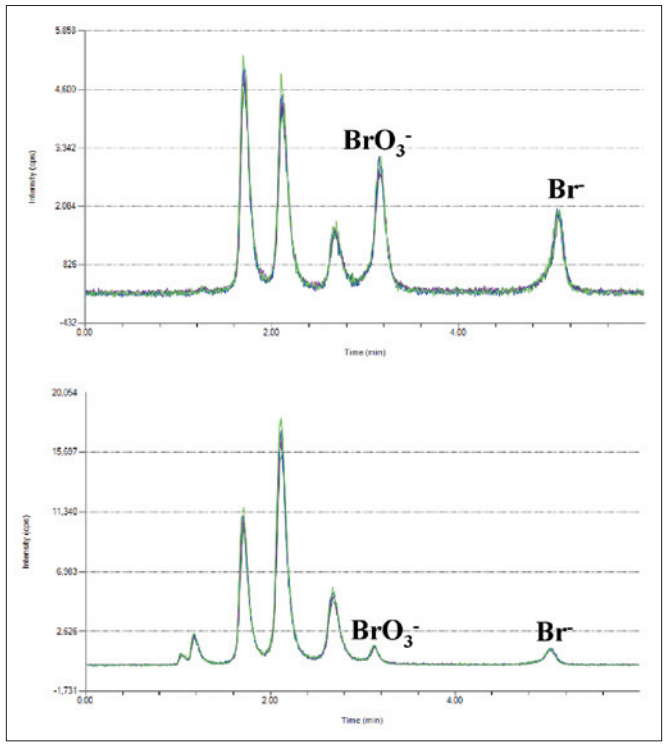

Figure 7. DRC-mode chromatograms of the samples in Figure 6, obtained with a gradient LC method.

A gradient HPLC separation scheme was then developed to separate the additional compounds. Figure 6 shows chromatograms of the two water samples shown in Figure 4, but with a gradient HPLC method. The additional peaks in Figure 6 (as compared to Figure 4) confirm the presence of additional Br-containing species and indicate the need to perform a gradient separation to obtain true BrO_3^- concentrations. Further confirmation is presented in Figure 7, which shows the DRC-mode chromatograms for the gradient separation of the samples in Figure 6. Table 5 shows the quantitative results of bromate and bromide for the water samples analyzed under the gradient method.

When comparing the results of the isocratic and gradient separations in Tables 4 and 5, a few waters showed higher bromate concentrations with the isocratic method than the gradient method (Thailand 1 and 3, China 3 and 4). The reason for this is incomplete separation of unidentified species which co-elute with bromate with the isocratic method. Although this incomplete separation leads to false high results, the combination of the isocratic and gradient methods could be used in tandem, with the isocratic method used for rapid screening, and the gradient method used for samples showing bromate concentrations above a pre-defined level. Since both methods use the same column and mobile phase components, switching between methods is simple.

It should be noted that no work was done to identify the additional bromine species. This could be accomplished with HPLC/ICP-MS by analyzing other known bromine-containing compounds and and matching retention times with the unidentified bromine compounds. Another option would be to use LC/MS, which might allow the unknown species to be identified by examining the fragmentation pattern of the compounds. However, a main limitation of LC/MS is it's low-sensitivity, relative to HPLC/ICP-MS.

Table 4. **Quantitative Determination of Bromide and Bromate in Water Samples Over Four Days (All units in µg/L).**

Sample	Day 1		Day 2		Day 3		Day 4	
	BrO_3^-	Br^-	BrO_3^-	Br^-	BrO_3^-	Br^-	BrO_3^-	Br^-
Australia	—	39.9	—	40.8	—	40.4	—	42.6
Brazil	—	6.91	—	6.53	—	7.02	—	7.40
Spain	—	23.0	—	22.7	—	22.4	—	22.6
Thailand-1	75.2	12.5	82.9	11.3	82.3	9.74	24.4	8.35
Thailand-2	83.6	—	81.1	—	78.1	—	79.3	—
Thailand-3	39.5	10.2	42.8	9.21	42.1	8.79	44.3	8.43
U.S.-1	—	10.7	—	10.0	—	10.1	—	8.36
U.S.-2	—	233	—	237	—	228	—	231
China-1	20.0	6.72	22.9	7.23	22.0	5.14	19.8	6.13
China-2	17.2	12.4	17.2	12.6	17.0	13.4	15.0	12.8
China-3	14.2	416	23.0	422	23.0	400	26.1	421
China-4	99.2	13.9	124	15.4	120	43.4	113	41.5
China-5	—	68.1	—	67.1	—	66.5	—	66.9

All samples are bottled waters, except China 3 and China 5 which are tap waters.

Table 5. **Quantitative Determination of Bromide and Bromate in Water Samples with the Gradient HPLC Method (All units in µg/L).**

Sample	BrO_3^-	Br^-
Australia	—	38.1
Brazil	—	7.94
Spain	—	23.5
Thailand-1	37.4	17.3
Thailand-2	76.0	—
Thailand-3	3.03	9.36
U.S.-1	—	8.79
U.S.-2	—	224
China-1	20.8	7.41
China-2	16.1	11.9
China-3	—	380
China-4	14.0	11.8
China-5	—	64.6

All samples are bottled waters, except China 3 and China 5 which are tap waters.

Conclusion

This work has demonstrated a rapid, robust method for separating and measuring bromide and bromate in drinking waters. The separations are accomplished in less than three minutes and proved to be repeatable from injection to injection and over several days. For those waters containing additional bromine-containing species, a gradient HPLC method was established. Taken together, the isocratic separation scheme can serve as a rapid screening method; those samples which contain additional bromine species can then be analyzed by the longer gradient method. If lower levels need to be measured, the injection volume of the HPLC autosampler can be increased.

References

1. Perrone, P.A., Reuter, W.M., Neubauer, K.R., Bosnak, C.P., Hall, G.A., Grosser, Z.A, ***Bromine Speciation by HPLC/ICP-MS*** (Application Note), 2005, PerkinElmer, Inc.

2. Anicich, V.G. ***An Index of the Literature for Bimolecular Gas Phase Cation-Molecule Reaction Kinetics,*** 2003, National Aeronautics and Space Administration.

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