

Application News

No. C152

Liquid Chromatograph Mass Spectrometry

Analysis of Bromate in Tap Water Using a Triple Quadrupole LC/MS/MS (2)

Bromate in tap water is generated by advanced water treatment processes such as ozone disinfection in the process of water purification. Potassium bromate has been classified by the IARC (1999) as a Group B2 substance which is possibly carcinogenic to humans, and was added to the water quality standards of Japan in 2004 by the Ministry of Health, Labour and Welfare (MHLW). Upon its addition, ion chromatography post-column absorption spectroscopy was designated as the testing method for bromate. (Annex table 18 of "Method Determined by the Minister of Health, Labour and Welfare on the Basis of the Ordinance Provisions Relating to Water Quality Standards", Notification No. 261 issued by the MHLW in 2003)

In December 2016, the MHLW gathered opinions for partial amendment of this method, and as given in annex table 18-2 "Liquid Chromatography-Mass Spectrometry", mass spectrometry was proposed as a new testing method (proposal) for bromate.

In Application News No. C144, we introduced an examination of analysis conditions using an anion exchange column according to this newly proposed testing method. In this article, we introduce the results of our examination of LC/MS/MS analysis utilizing a mixed-mode column (multi-mode column) as a new LC separation mode.

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■ Examination of Conditions for Analysis Using a Mixed-Mode Column

Bromate is difficult to retain with reversed-phase LC, which is widely used for LC/MS/MS, because it is a highly polar compound. Therefore in this research, we examined conditions such as mobile phases based on the newly proposed testing method, and established analysis conditions (Table 1) utilizing a mixed-mode column. A mixed-mode column is capable of multiple separation modes. This time, we used a mixed-mode column with a reversed phase and capable of anion exchange and cation exchange modes.

Fig. 1 shows a chromatogram of the standard solution of 0.001 mg/L, which is equivalent to one-tenth the water quality criterion. With these analysis conditions, bromate was eluted at 2.7 min, exhibiting good retention and a good peak shape.

Fig. 2 shows a six-point calibration curve for concentrations ranging from 0.0005 mg/L to 0.01 mg/L. Favorable linearity was achieved with a correlation coefficient (R) of 0.9995 and a coefficient of determination (R²) of 0.9990.

Table 1 Analysis Conditions

Column	: Imtakt Scherzo SS-C18 (150 mm L. × 2.0 mm I.D., 3 μm)
Mobile phases	: A) 200 mmol/L ammonium acetate - water containing 0.5 % acetic acid B) acetonitrile
Time programs	: B conc. 90 % (0 - 7 min) - 5 % (7.01 - 12 min) - 90 % (12.01 - 17 min)
Flow rate	: 0.3 mL/min
Column temp.	: 40 °C
Injection Volume	: 10 μL
Probe Voltage	: -1 kV (ESI-Negative)
DL temp.	: 100 °C
Block Heater temp.	: 300 °C
Interface temp.	: 300 °C
Nebulizing gas flow	: 2 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 10 L/min
MRM transition	: Bromate ion m/z 129.00>112.95

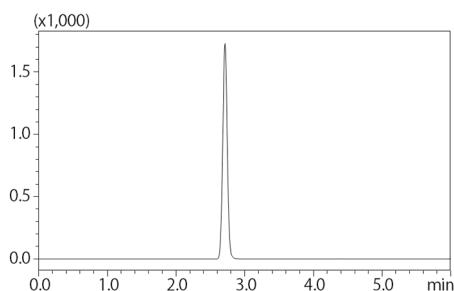


Fig. 1 Chromatogram of Standard Solution of 0.001 mg/L Bromate

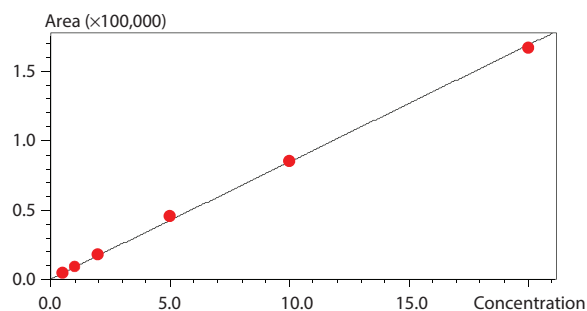


Fig. 2 Calibration Curve of Bromate (0.0005 mg/L to 0.01 mg/L)

Verifying Separation from Anion Impurities in Tap Water

Bromate in tap water can be selectively detected by MRM measurement with LC/MS/MS. However, anions such as sulfate ions also exist in the tap water sample. By separating the chromatograms of such anion impurities from that of bromate, determination precision in tap water analysis can be improved. This necessity can also be verified by the statement in the liquid chromatography-mass spectrometry method (proposal): When the water for testing includes a high concentration of sulfate ions, set analysis conditions under which sulfate ions elute from the separation column.

Fig. 3 shows the chromatograms obtained by adding bromate to a tap water sample (from Kanagawa Prefecture), and monitoring bromate together with anion impurities. Sulfate ions, chloride ions, nitrate ions, and chlorate ions in the tap water are also retained and eluted, indicating that bromate is separated from these ions.

With these analysis conditions utilizing a mixed-mode column, sulfate ions, etc., are eluted using an aqueous mobile phase containing 200 mmol/L of ammonium acetate as given in the newly proposed testing method. In general, usage of mobile phases with high salt concentrations in the MS may require maintenance more frequently than usual. Therefore with these analysis conditions, the flow line following the separation column is switched from the MS side to the drain side by a switching valve for the retention time range from 7 min to 15 min in which the proportion of the aqueous mobile phase is increased. By doing so, the mobile phase containing high salt concentrations will be discharged, enhancing robustness with regard to system maintenance.

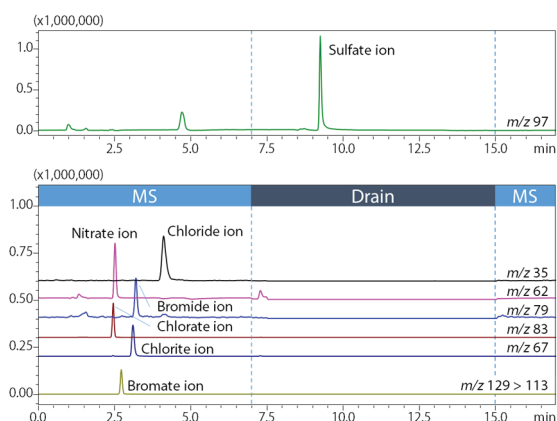


Fig. 3 Chromatograms of Anion Impurities and Bromate in Tap Water

Spike-and-Recovery Test with Tap Water

A spike-and-recovery test of bromate was performed using tap water (from Kanagawa Prefecture). Tap water was measured after being spiked with 0.01 mg/L bromate, which is the water quality criterion, and with 0.001 mg/L bromate, which is a concentration one-tenth the water quality criterion. The obtained chromatograms showed no significant disturbance originating from impurities in tap water (Fig. 4).

Table 2 shows the accuracy and precision calculated from the analysis results of these samples. With both spiking concentrations, the standard given in the validation guideline (notification issued by the MHLW in Sept. 2012) was fulfilled.

With the mixed-mode column utilized in this examination, we confirmed that bromate in a tap water sample can be quantitated without pretreatment down to a concentration of 0.001 mg/L, which is one-tenth the criterion, likewise the analysis introduced in Application News No. C144 using an anion exchange column.

Unlike the current ion chromatography post-column absorption spectroscopy method, this analysis method does not require preparation of a reagent, and is therefore expected to improve the efficiency of water quality testing and contribute to reducing the burden of tests.

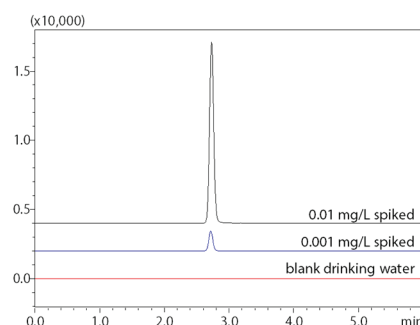


Fig. 4 Chromatograms of Blank Tap Water and Tap Water Spiked with Bromate Standard

Table 2 Spike-and-Recovery Test Results of Bromate (n = 5)

Spiked Conc. mg/L	Accuracy %	Precision %RSD
0.01	92.4	2.0
0.001	86.5	5.5