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Application News

High Performance Liquid Chromatograph

Determination of Disinfection By-Products in Drinking Water According to EPA Method 326.0

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User Benefits

- ◆ The analysis using Nexera™ lite inert system can be conducted in a measurement time of approximately 15 min.
- ◆ Disinfection byproducts (chlorites, bromates, bromides, chlorates) with low concentrations can be analyzed reproducibly using an electrical conductivity detector.
- ◆ Bromate ion can be detected with a post column reaction after separation of column using a UV detector, enabling for selective detection and quantification.

■ Introduction

Disinfection is typically employed for water purification and the protection of public health. However, certain by-products generated from chemical disinfection processes can be harmful. For example, it is known that the chlorine dioxide disinfection process generates chlorite and chlorate, which are inorganic oxyhalide disinfection by-products (DBPs), and hypochlorite treatment may also produce chlorate, another DBP. It is necessary to appropriately manage these harmful DBPs.

Most countries establish limits and regulations for disinfection by-products (DBPs) in drinking water and environmental water. In the United States, DBPs are regulated by the Environmental Protection Agency (EPA) under Method 326.0[1)] for drinking water.

This application news introduces an example of DBPs analysis in drinking water in accordance with EPA Method 326.0, utilizing the Shimadzu Nexera lite inert system. Fig. 1 shows the flow path diagram of the DBPs analysis system. The four types of DBPs were separated using a Shim-pack™ IC-SA3 and analyzed with an electrical conductivity detector (CDD). Furthermore, bromate was detected through a post-column reaction followed by UV detection, achieving high-sensitivity analysis.

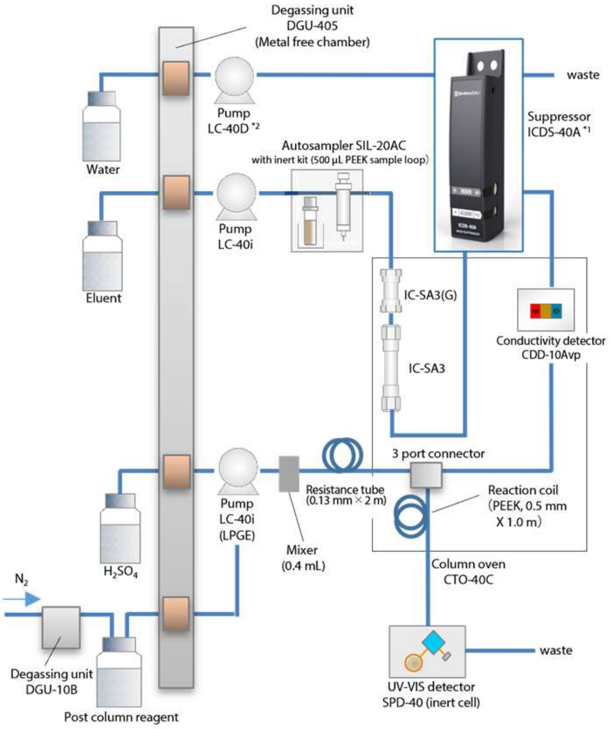

Fig. 1 Flow Path Diagram of DBPs Analysis System

*1 To improve the detection sensitivity of electrical conductivity, an electrically regenerable suppressor was introduced.
*2 A pump LC-40D was used for the liquid supply side of the regenerable liquid in the electrically regenerable suppressor (other pumps can also be used as substitutes).

■ EPA Method 326.0

This method outlines the determination of inorganic oxyhalide disinfection by-product anions in reagent water, surface water, groundwater, and finished drinking water. The analytes include chlorite (ClO_2^-), bromate (BrO_3^-), bromide (Br^-) and chlorate (ClO_3^-).

The initial part of the analysis follows the procedures outlined in EPA Method 300.1, Part B. The DBPs are quantified using conductivity detection following column separation and suppression of the eluent.

To enhance low-level detection of bromate, the suppressed effluent from the conductivity detector is combined with an acidic solution of potassium iodide (KI) containing a catalytic amount of molybdenum VI to produce hydroiodic acid (HI). Subsequently, bromate reacts with iodide to form the tri-iodide ion (I_3^-) which is measured by its UV absorption at 352 nm.

The reactions are as follows:

$$BrO_3^- + 3I^- + 3H^+ \Leftrightarrow 3HOI + Br^-$$
$$3HOI + 3I^- + 3H^+ \Leftrightarrow 3I_2 + 3H_2O$$
$$3I_2 + 3I^- \Leftrightarrow 3I_3^-$$

In EPA Method 326.0, the addition of dichloroacetic acid (DCA) as a surrogate solution is specified, and DCA is added to all samples to achieve a final concentration of 1 mg/L. Additionally, ethylenediamine (EDA) is incorporated to suppress the degradation of DBPs.

■ Analytical Conditions

Table 1 Analysis Conditions of Nexera lite inert

System	:	Nexera lite inert
Column	:	Shim-pack IC-SA3 (4.0 mm × 250 mm I.D., 5 µm)
Guard Column	:	Shim-pack IC-SA3 (G)
Eluent	:	3.6 mmol/L Na_2CO_3
Flowrate of Eluent	:	0.8 mL/min
Post column Reagent	:	0.16 mol/L KI 0.035 mmol/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
Concentration of H_2SO_4	:	0.5 mol/L
Flowrate of LPGE	:	0.4 mL/min (Post column reagent : H_2SO_4 = 50 : 50)
Flowrate of Suppressor	:	0.8 mL/min
Reaction Coil	:	0.5 mm × 1 m
Injection Volume	:	300 µL
Temp. of Column Oven	:	40 °C
Degassing Unit for Post Column Reagent	:	DGU-10B*[1]
Gas for Degassing Unit	:	N_2
Vial	:	Shimadzu Vial, LC, 4 mL, Polypropylene*[2]
Detection	:	1. Conductivity 2. UV-VIS (352 nm, inert cell)

*1 The helium degas unit was used instead.
*2 P/N: 228-31537-91

■ Analysis of Standard Samples

The Quality Control (QC) in EPA Method 326.0 requires prior confirmation of reagent blank, linearity of calibration curve, accuracy and precision of calibration point to evaluate the analytical method and system performance. The concentrations of standard samples for each analyte are shown in Table 2.

Table 2 Concentration of Calibration Samples

Analyte (µg/L)		**STD 1**	**STD 2**	**STD 3**	**STD 4**	**STD 5**	**STD 6**
CDD	ClO_2^-	5	10	25	50	100	200
	BrO_3^-	5	10	25	50	100	200
	Br^-	5	10	25	50	100	200
	ClO_3^-	5	10	25	50	100	200
UV	BrO_3^-	0.5	1	2	5	10	

In the analyses using the electrical conductivity detector, the calibration curve was created with 6 points between 5-200 µg/L in accordance with EPA requirements. The coefficient of correlation (r^2) for the calibration curve was more than 0.999. Fig. 2 shows the chromatogram of the 50 µg/L standard sample using the electrical conductivity detector.

In the analyses using the UV detector, the calibration curve was created with 5 points between 0.5-10 µg/L in accordance with EPA requirements. The coefficient of correlation for the calibration curve was higher than 0.9999. Fig. 3 shows the chromatogram of the 5 µg/L standard sample using the UV detector.

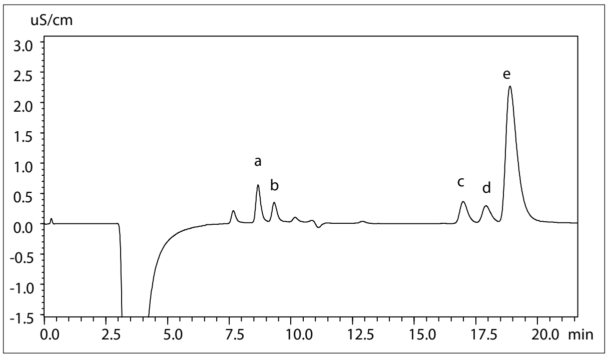

Fig. 2 Chromatogram of 50 µg/L Standard Sample
(Electrical Conductivity Detector)
(a: ClO_2^-, b: BrO_3^-, c: Br^-, d: ClO_3^-, e: DCA)

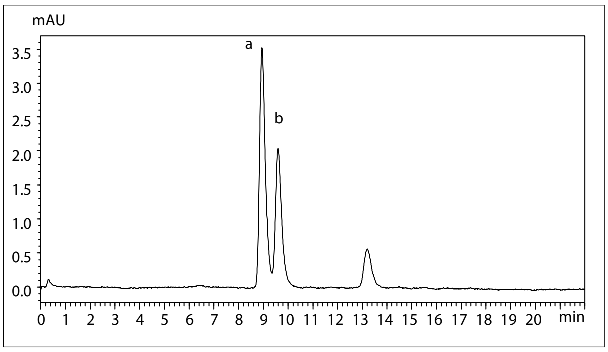

Fig. 3 Chromatogram of 50 µg/L Standard Sample (UV Detector)
(a: ClO_2^-, b: BrO_3^-)

Seven replicated analyses were conducted at three points on the calibration curve, the low-level, the mid-level, and the high-level concentrations, to confirm average concentration, precision and accuracy. Table 3 and Table 4 shows these results. Both the relative standard deviation (%RSD) and the mean recoveries of true values met EPA requirements. Moreover, a blank sample was analyzed immediately after the highest standard sample, which confirmed that no carry-over occurred.

Table 3 Accuracy and Precision of Standard Samples
(Electrical Conductivity Detector) (n = 7)

Detector		Electrical conductivity			
Analyte		ClO_2^-	BrO_3^-	Br^-	ClO_3^-
5 µg/L	Average (µg/L)	5.3	5.4	5.6	5.5
	Accuracy (%)	106	108	113	110
	%RSD	2.5	1.4	3.5	4.1
25 µg/L	Average (µg/L)	26	26	25	24
	Accuracy (%)	103	103	100	95
	%RSD	0.33	0.41	1.3	3.6
100 µg/L	Average (µg/L)	100	100	101	102
	Accuracy (%)	100	100	101	102
	%RSD	0.29	0.23	0.48	1.2

Table 4 Accuracy and Precision of Standard Samples (UV detector) (n = 7)

Detector		UV
Analyte		BrO_3^-
0.5 µg/L	Average (µg/L)	0.53
	Accuracy (%)	106
	%RSD	4.8
2 µg/L	Average (µg/L)	2.0
	Accuracy (%)	102
	%RSD	2.1
10 µg/L	Average (µg/L)	9.9
	Accuracy (%)	99
	%RSD	0.30

■ Detection Limit and Quantitation Limit

The detection limit (DL) was calculated as (t) × (s) by preparing the DL standard solution at the estimated concentration of DL, as prescribed in EPA Method 326.0, and conducting seven replicated analyses. Table 5 shows the calculation formula, and the calculated DLs are shown in Table 6.

Additionally, based on the standard deviation of the bromate ion obtained from seven replicated analyses, the limit of quantification (LOQ) was calculated to be 0.3 µg/L. This value satisfies the limit of 3 µg/L established for bottled mineral water and spring water treated with ozone, as published in Europe[2)].

Table 5 DL Calculation Formula

$$\mathbf{DL = (t) \times (s)}$$

t = Student's t value for n-1 degrees of freedom at the 99 % confidence level;
t = 3.143 for six degrees of freedom
s = standard deviation of the replicated analyses

Table 6 The Standard Deviation and Detection Limit of Each Analyte (n = 7)

Detector	Electrical conductivity				UV
Analyte	ClO_2^-	BrO_3^-	Br^-	ClO_3^-	BrO_3^-
Concentration µg/L	5	5	5	5	0.5
Standard deviation	0.15	0.16	0.23	0.31	0.025
DL µg/L	0.4	0.5	0.7	1.0	0.08

■ Analysis of Samples and Spike Recoveries

Six types of drinking water were analyzed independently on two occasions, in accordance with EPA Method 326.0. For the pretreatment of the drinking water samples, DCA and EDA were added to each sample.

As an example of a high matrix sample, high ionic strength water (HIW) was prepared as described in EPA Method 326.0. The HIW was created from reagent water fortified with common anions: chloride at 100 mg/L, carbonate at 100 mg/L, nitrate at 10.0 mg/L, phosphate at 10.0 mg/L, and sulfate at 100 mg/L.

In the spike recovery test, the six types of drinking water spiked with the standard sample were prepared and analyzed. For the analysis using the electrical conductivity detector, 50 µg/L of each standard solution was spiked into the drinking water samples. For the UV detector analysis, which is used for high sensitivity detection of bromate, a 2 µg/L bromate standard solution was spiked into the drinking water samples.

The spike recoveries and relative percent difference (RPD) were calculated in accordance with EPA Method 326.0. The calculation formulas are shown in Tables 7 and 8. Fig. 4-1 and Fig. 4-2 present examples of the analysis results.

Table 7 The Formula of Spike Recovery

$$\mathbf{R} = \frac{C_F - C}{F} \times \mathbf{100}$$

R = Percent recovery
CF = Fortified sample concentration
C = Sample background concentration
F = Concentration equivalent added to sample

Table 8 The Formula of Relative Percent Difference

$$\mathbf{RPD} = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} \mathbf{{*}100}$$

FD1 = The first duplicate measurement
FD2 = The second duplicate measurement

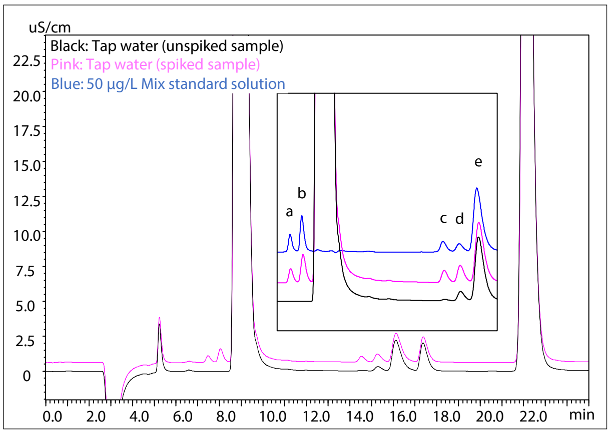

Fig. 4-1 The Chromatograms of Tap Water and Spiked Tap Water (Electrical Conductivity Detector)(a: ClO_2^-, b: BrO_3^-, c: Br^-, d: ClO_3^-, e: DCA)

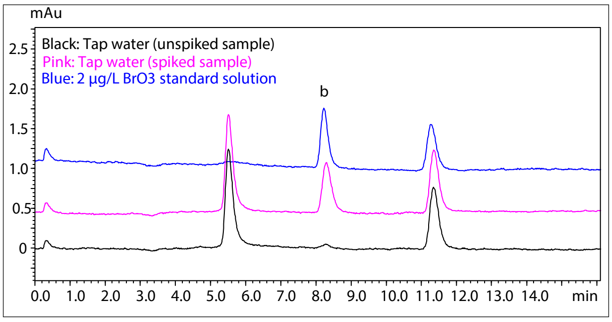

Fig. 4-2 The Chromatograms of Tap Water and Spiked Tap Water (UV Detector) (b: BrO_3^-)

The presence of high cation concentrations was confirmed in certain types of drinking water samples. In these samples, we observed peak broadening of chlorite and bromate in both the electrical conductivity detector and the UV detector was observed. It could be hypothesized that this phenomenon is caused by the interference of cations such as calcium.

Here, for these samples, the Maxi-Clean cartridge IC-H (0.5 mL) was used. Prior to sample analysis, the interfering cations were eliminated as part of the pretreatment process. The results obtained from the pretreatment samples showed improved peak shapes for chlorite and bromate with both the electrical conductivity detector and the UV detector, with satisfactory spike recoveries.

Fig. 5-1 and 5-2 present examples of the analysis results without the use of the cartridge, while Fig. 6-1 and 6-2 illustrate the analysis results obtained with the cartridge.

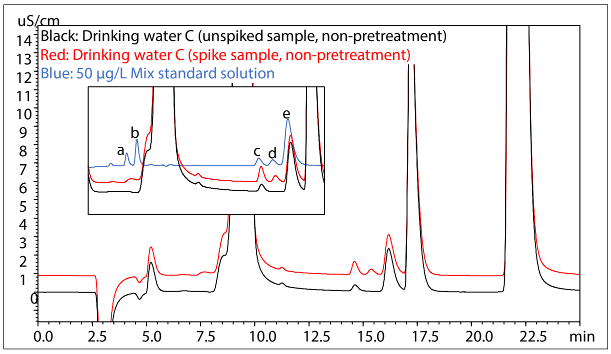

Fig. 5-1 The Chromatograms of Drinking Water C and Spiked Drinking Water C without Pretreatment (Electrical Conductivity Detector) (a: ClO_2^-, b: BrO_3^-, c: Br^-, d: ClO_3^-, e: DCA)

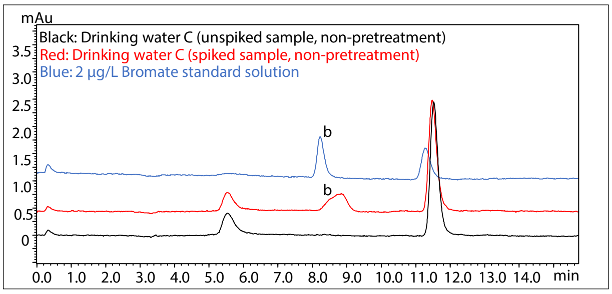

Fig. 5-2 The Chromatograms of Drinking Water C and Spiked Drinking Water C without Pretreatment (UV Detector) (b: BrO_3^-)

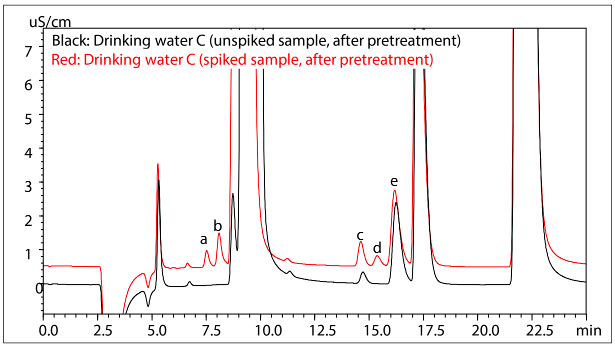

Fig. 6-1 The Chromatograms of Drinking Water C and Spiked Drinking Water C with Pretreatment (Electrical Conductivity Detector) (a: ClO_2^-, b: BrO_3^-, c: Br^-, d: ClO_3^-, e: DCA)

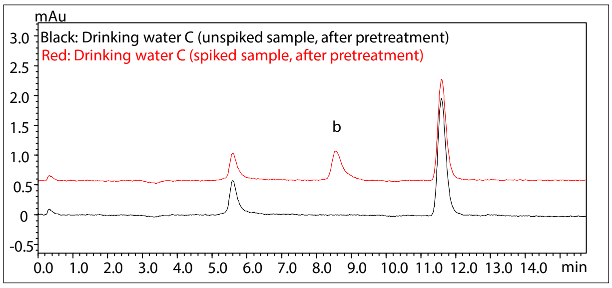

Fig. 6-2 The Chromatograms of Drinking Water C and Spiked Drinking Water C with Pretreatment (UV Detector) (b: BrO_3^-)

The analysis results for the six types of drinking water, as well as the spike recovery rates and PRD, are presented in Table 9. The spike recovery rates for all components were within the range of 84 to 106 %, meeting the required range of 75 to 125 % specified in the QC criteria of EPA Method 326.0. The recovery of the DCA surrogate was also within the required QC range of 90 to 115 %. Additionally, all PRD values were below 10 %, thereby satisfying the requirements set forth in the QC criteria.

Table 9 The Analysis Results and Spike Recoveries of Six Types of Drinking Water (n = 2)

Sample		Drinking Water A				Tap Water				HIW			
Usage of Cartridge		No				No				No			
Analytes		Conc. (µg/L)	Spiked Conc. (µg/L)	RPD of Spiked Sample	Spike Recovery	Conc. (µg/L)	Spiked Conc. (µg/L)	RPD of Spiked Sample	Spike Recovery	Conc. (µg/L)	Spiked Conc. (µg/L)	RPD of Spiked Sample	Spike Recovery
CDD	ClO_2^-	N.D.*1	49	0.21	99 %	N.D.	49	0.43	98 %	N.D.	43	1.2	86 %
	BrO_3^-	N.D.	48	0.09	97 %	N.D.	48	0.64	97 %	N.D.	43	1.4	86 %
	Br^-	9.5	60	0.47	100 %	7.78	58	0.78	100 %	N.D.	52	1.5	104 %
	ClO_3^-	N.D.	52	0.34	104 %	58.6	106	0.75	94 %	N.D.	50	2.6	99 %
	DCA	1027			103 %	1027			103 %				
UV	BrO_3^-	N.D.	1.9	5.0	97 %	N.D.	2.1	2.8	103 %	N.D.	1.96	6.4	98 %
Sample		Drinking Water B				Drinking Water C				Drinking Water D			
Usage of Cartridge		Yes				Yes				Yes			
Analytes		Conc. (µg/L)	Spiked Conc. (µg/L)	RPD of Spiked Sample	Spike Recovery	Conc. (µg/L)	Spiked Conc. (µg/L)	RPD of Spiked Sample	Spike Recovery	Conc. (µg/L)	Spiked Conc. (µg/L)	RPD of Spiked Sample	Spike Recovery
CDD	ClO_2^-	N.D.	47	5.6	94 %	N.D.	46	0.18	92 %	N.D.	42	0.10	84 %
	BrO_3^-	N.D.	51	5.4	102 %	N.D.	48	0.18	96 %	N.D.	51	1.2	101 %
	Br^-	8.65	59	0.44	100 %	44.2	93	0.08	98 %	31.4	81	0.07	100 %
	ClO_3^-	N.D.	53	1.6	106 %	N.D.	53	0.33	105 %	N.D.	53	0.09	105 %
	DCA	1009			101 %	1014			101 %	1019			102 %
UV	BrO_3^-	N.D.	2.0	2.0	100 %	N.D.	1.96	5.5	98 %	N.D.	2.1	0.14	103 %

*1: N.D. < DL

■ Conclusion

In this Application News, the analysis of disinfection by-products in drinking water in accordance with EPA Method 326.0, utilizing the Nexera lite inert system was introduced. Four types of DBPs were separated using the Shim-pack IC-SA3 and analyzed with an electrical conductivity detector. Additionally, the bromate ion was subjected to post-column reaction after column separation, enabling high-sensitivity analysis with a UV detector. The analysis demonstrated good reproducibility and satisfactory results in the spike recovery tests.

Furthermore, in the analysis using the UV detector, the quantification limit for bromate ions was calculated to be 0.3 µg/L. This value satisfies the limit of 3 µg/L established for bottled mineral water and spring water treated with ozone, as published in Europe.

<References>

1) Method 326.0: Determination of Inorganic Oxyhalide Disinfection By-Products in Drinking Water Using Ion Chromatography Incorporating the Addition of a Suppressor Acidified Postcolumn Reagent for Trace Bromate Analysis, Version 1.0

2) Commission Directive 2003/40/EC. (Referenced in 2025/09)

<Related Applications>

1. Analysis of inorganic anions in tap water according to EPA Method 300.1 using Ion Chromatography, Application News 01-00487

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› Nexera lite inert

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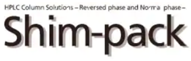

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