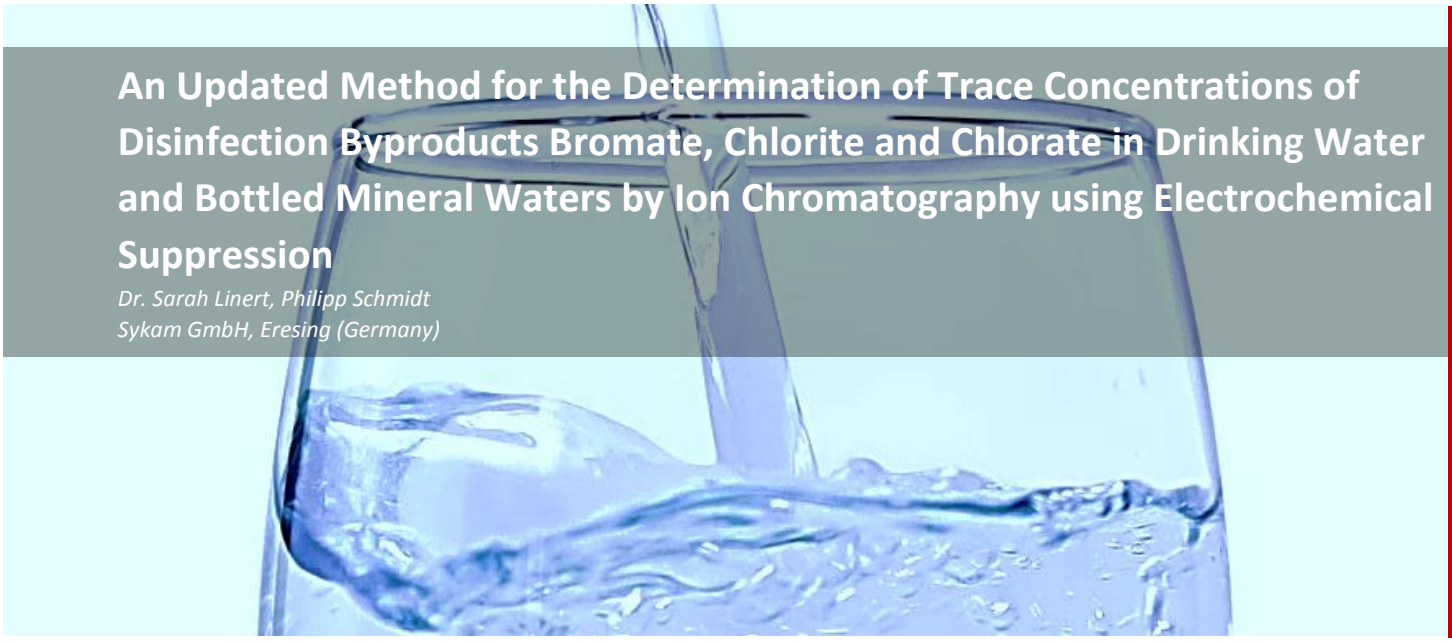

An Updated Method for the Determination of Trace Concentrations of Disinfection Byproducts Bromate, Chlorite and Chlorate in Drinking Water and Bottled Mineral Waters by Ion Chromatography using Electrochemical Suppression

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Sykam Application Update 15

Introduction

In most countries a secured drinking water supply from public water systems is a self-evident responsibility of public services. To ensure this, many water suppliers must treat their water using well established disinfection techniques due to contaminations with potentially dangerous microbes or other ingredients, which may cause adverse health effects after consumption. Commonly used disinfecting agents are chlorine, chlorine dioxide, chloramine or ozone.[1,2]

The chemical disinfectants used for water treatment may react with naturally occurring inorganic or organic compounds to form disinfection byproducts (DBPs), which cause undesired health effects after human consumption.[2] Chlorination, a technique that is still widely used today, can form trihalomethanes, haloacetic acids or chlorate,[3] and the treatment with chlorine dioxide or chloramine is known to produce the oxyhalide DBPs chlorite and chlorate.[4] To avoid taste and odor, often caused by treating drinking water with disinfectants, many suppliers instead use a treatment with ozone enriched air for disinfection. Ozone, as a strong oxidant, however reacts with the naturally occurring bromide to form bromate,[5] which has been identified as a potential carcinogen by the International Agency for Research on Cancer.[6] The World Health Organization (WHO) has estimated an excess lifetime cancer risk of 10^{-4}, 10^{-5} and 10^{-6} by drinking water containing bromate at levels of 20, 2 and 0.2 µg/L respectively.[7] Therefore, many authorities like the European Commission (EC) or the US Environmental Protection Agency (US EPA) have established maximum contaminant levels (MCL) for DBPs in drinking and bottled mineral waters. The European Union (EU Directive 98/83/EC)[8] as well as the EPA set the maximum contaminant level for bromate to 10 µg/L.[9] Additionally, the EC established a lower MCL of 3 µg/L for mineral and spring waters treated with ozone.[10] Chlorite and Chlorate are limited to 0.25 mg/L in the EU for drinking water and 0.70 mg/L for treated drinking water, especially if chlorine dioxide is used as disinfectant.[8] The EPA set the MCL for chlorite to 1000 µg/L under the Disinfectants/Disinfection Byproducts (D/DBP) Stage 1 Rule.[9]

The need to meet the requirements set by the EU or US authorities regarding the contamination limits of DBPs like bromate, chlorite and chlorate has led to the publication of a series of regulatory analytical methods for the determination of DBPs in drinking water. Among these methods, Ion Chromatography (IC) has been established as the most common technique, as it allows reliable quantification of DBPs. The US EPA has published EPA Method 300.1,[11] which employs suppressed conductivity measurement for the analysis of DBPs in drinking water and the European counterpart to this regulatory method is the EN ISO 15061.[12] Additional techniques for the determination of low µg/L levels of bromate include IC with post-column derivatization and UV detection (EPA Methods 317.0 and 326.0) or IC/ICP-MS (EPA Method 321.8).[13]

In our Application Note AN12 a method for the determination of DBPs is presented. This method uses two columns of the type Sykam A10 (250 x 4.0 mm) in series to achieve the desired resolution and detection limits of 2.64 µg/L for bromate, 1.09 µg/L for chlorite and 2.59 µg/L for chlorate. The DBPs can easily be quantified down to the concentration levels of their respective MCLs, as defined by the EC[8,10] and the EPA.[9] Next to the disinfection byproducts chlorite, chlorate, bromate and its precursor bromide, the common inorganic anions fluoride, chloride, nitrite, nitrate, phosphate and sulfate can also be resolved and determined in the same run using the described method.

However, with this method it is not possible to perform a simultaneous determination of cations, due to the space requirements of the two A10 columns, which do not allow the placement of an additional cation separation column on the column oven.

SYKAM CHROMATOGRAPHY

In this Application Update "Sykam AU15", a method for the determination of DBPs and bromide in drinking water and bottled mineral waters by IC, using suppressed conductivity measurement with electrochemical suppression, is described that requires only one Sykam A10 anion separation column with an AGC-06 guard column. This method is slightly shorter than the method described in AN12 and allows simultaneous determination of cations. The results of the quality control parameter tests for this method are comparable to those of the method described in AN12. The testing requirements of the EPA 300.1, Revision 1.0 (Part B)[11] as well as EN ISO 15061:2001-12[12] and ASTM D6581-18[14] are met since the method was validated under consideration of the protocols described therein.

Equipment

The application update Sykam AU15 was designed for the use of a Sykam S151-AG+ IC system for single channel anion detection consisting of the following components:

- S150+ Ion Chromatography Module including column oven, single-channel conductivity detector and electrochemical self-regenerating anion suppressor module
- S1130 Quaternary Gradient Pump (PEEK) including 4-channel degasser
- S5300 Automatic Sample Injector with S6115 injection valve (PEEK)
- S7150 Reagent Organizer with four eluent bottles (2 x 2000 mL, 2 x 1000 mL)
- Clarity advanced chromatography software for Windows (DataApex)

The use of a S1130 isocratic pump instead of a S1130 gradient pump is also possible as well as the use of a S6120 manual injection valve instead of a S5300 Automatic Sample Injector. Alternatively, the S153-AG+ IC system for dual channel analysis can be used for this application, if the system is run in single channel mode for anion detection.

Reagents and Standards

All reagents for eluent and standard preparation should be of analytical grade (ACS, p.a.) or better. We recommend the use of the following commercially available reagents. Alternatively, other reagents and standards can be used, if they are of comparable or better purity.

- Deionized water, Type I reagent grade, 0.1 µS/cm conductivity (10 kΩ/cm resistivity) or better
- Sodium carbonate (Na_2CO_3, anhydrous, for analysis, ACS, ISO, Reag. Ph Eur), Merck (1.06393)
- Sodium bicarbonate ($NaHCO_3$, for analysis, ACS, Reag.Ph Eur), Merck (1.06329)
- Ethylene diamine (ReagentPlus, puriss. p.a., ≥99.5% (GC), Sigma-Aldrich (03550).
- Bromide standard solution 1000 mg/L (traceable to SRM from NIST NaBr in H_2O 1000 mg/L Br Certipur®), Merck (1.19896)
- Bromate standard solution 1000 mg/L in H_2O (traceable to SRM from NIST), Sigma-Aldrich (78476)
- Chlorite standard solution 1000 mg/L in H_2O, Sigma-Aldrich (ICS-006)
- Chlorate standard solution 1000mg/L in H_2O (traceable to SRM from NIST), Sigma-Aldrich (73166)

For the preparation of the artificial drinking water sample, or in case of additional quantification of the standard inorganic anions, the following standard solutions can be used:

- Fluoride standard solution 1000 mg/L (traceable to SRM from NIST NaF in H_2O 1000 mg/L F Certipur®), Merck (1.19814)
- Chloride standard solution 1000 mg/L (traceable to SRM from NIST NaCl in H_2O 1000 mg/L Cl Certipur®), Merck (1.19897)
- Nitrite standard solution 1000 mg/L (traceable to SRM from NIST $NaNO_2$ in H_2O 1000 mg/L NO_2 Certipur®), Merck (1.19899)
- Nitrate standard solution 1000 mg/L (traceable to SRM from NIST $NaNO_3$ in H_2O 1000 mg/L NO_3 Certipur®), Merck (1.19811)
- Phosphate standard solution 1000 mg/L (traceable to SRM from NIST KH_2PO_4 in H_2O 1000 mg/L PO_4 Certipur®), Merck (1.19898)
- Sulfate standard solution 1000 mg/L (traceable to SRM from NIST Na_2SO_4 in H_2O 1000 mg/L SO_4 Certipur®), Merck (1.19813)

If the 1000 mg/L standard solutions are freshly prepared from sodium or potassium salts, we recommend the use of the following analytical or ACS reagent grade chemicals. Alternatively, other chemicals of comparable or better purity can be used. Anhydrous sodium or potassium salts should be used for the preparation of standard solutions since they can be dried in a vacuum oven if necessary. The chlorite standard solution should not be prepared from crystalline material, since chlorites as crystalline compounds are only available with an assay of approx. 80%.

- Sodium bromide (NaBr, ACS reagent, ≥99.0%), Sigma-Aldrich (310506)
- Sodium bromate ($NaBrO_3$, for synthesis, ≥99.0%), Sigma-Aldrich (8.14368)
- Sodium chlorate ($NaClO_3$, ACS reagent, ≥99.0%), Sigma-Aldrich (403016)
- Sodium fluoride (NaF, for analysis EMSURE, Reag. Ph Eur), Merck (1.06449)
- Sodium chloride (NaCl, for analysis EMSURE, ACS, ISO, Reag. Ph Eur), Merck (1.06404)
- Sodium nitrite ($NaNO_2$, for analysis EMSURE, ACS, Reag. Ph Eur), Merck (1.06549)
- Sodium nitrate ($NaNO_3$, for analysis EMSURE, ACS, ISO, Reag. Ph Eur), Merck (1.06537)
- Potassium dihydrogen phosphate (KH_2PO_4, for analysis EMSURE, ISO), Merck (1.04873)
- Sodium sulfate (Na_2SO_4, anhydrous, for analysis EMSURE, ACS, ISO, Reag. Ph Eur), Merck (1.06649)

Samples

For the method validation, two drinking water samples from local municipal water suppliers and four commercially available bottled mineral waters were analyzed (Table 1).

Table 1. List of samples analyzed.

No.	Name
1	Drinking Water (Milbertshofen, Munich)
2	Drinking Water (Penzberg)
3	Mineral Water 1
4	Mineral Water 2
5	Mineral Water 3
6	Mineral Water 4

The local municipal water suppliers, as well as the companies selling the bottled mineral waters, obtain their drinking water from deep ground water sources. None of the samples is treated with disinfecting agents like chlorine, chlorine dioxide, chloramine or ozone and therefore none of the disinfection byproducts were detected in any of the samples as expected. None the less, resolution and recovery, as well as any other system performance parameter, were examined in this study. The two drinking water samples were collected in 1000 mL PE-bottles with screw caps and stored at 4 °C immediately after collection. The commercially available mineral waters were purchased and stored at 4 °C in their original bottles. All samples were also analyzed as laboratory duplicates.

Chromatographic Conditions

Columns:	Sykam A10 (250 x 4.0 mm), Analytical Column Sykam AGC-06 (50 x 4.6 mm), Guard Column
Eluent:	1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$
Flow Rate:	1.0 mL/min
Run Time:	46 min
Temperature:	35 °C
Injection Volume:	100 µL (full loop)
Detection:	Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor
Suppressor Current:	25 mA
Backpressure:	50 bar (725 psi)
Base Conductivity:	13 µS/cm
Noise:	<2 nS/cm

Preparation of Solutions and Reagents

Anion Standard Stock Solutions (1000 mg/L)

The standard stock solutions for the four analytes bromate, chlorite, chlorate and bromide can either be purchased from commercial sources as indicated in section “Reagents and Standards” or prepared from the respective anhydrous sodium and potassium salts, as indicated above, if commercial standard stock solutions are not available. If prepared from the respective sodium salt, the chlorite standard solution needs to be titrated and its assay calculated before use, since crystalline sodium chlorite is only available at an assay of 80%. The amounts needed for the preparation of 1000 mL of each standard stock solution are given in Table 2. If only selected anions are to be analyzed, the other standard solutions can be omitted. The 1000 mg/L standard stock solutions are stable for at least one month when stored at 4 °C.

Table 2. Preparation of standard stock solutions (1000 mg/L).

Analyte	Compound	Amount (g)
Bromate	Sodium bromate ($NaBrO_3$)	1.180
Chlorate	Sodium chlorate ($NaClO_3$)	1.276
Bromide	Sodium bromide (NaBr)	1.288

Ethylenediamine (EDA) Preservation Solution (100 mg/mL EDA)

2.8 mL of Ethylenediamine are diluted to 25 mL with deionized water. The preservation solution is stored at 4°C and needs to be prepared fresh monthly. In order to prevent redox processes, which may alter the concentrations of the disinfection byproducts, all samples and standards are treated with EDA by adding 50 mg/L preservation solution (e.g. 50 µL preservation solution/100 mL sample/standard).

Artificial Drinking Water (ADW)

All standards for the assessment of linearity, the Quality Control Standard (QCS), the External Control Standard (ECS) as well as the MDL-Calculation Standard are prepared in Artificial Drinking Water (ADW). This is meant simulate the interferences of other anions, which are usually contained in drinking water samples, on the determination of the analytes chlorite, bromate, chlorate and bromide. The composition of the artificial drinking water is selected to be close to that found in a typical drinking water sample. Table 3 summarizes the concentrations of the anions contained in ADW.

Table 3. Concentrations of the standard anions in artificial drinking water (ADW).

Analyte	ADW Composition (mg/L)
Fluoride	1.0
Chloride	50.0
Nitrite	0.1
Nitrate	30.0
Phosphate	0.1
Sulfate	50.0

Working Standard Solutions

All single and composite anion working standard solutions at concentrations lower than 1000 mg/L are prepared from the standard stock solutions and should be prepared fresh daily. For the preliminary estimation of the method detection limit (MDL), a series of mixed standards at concentrations of 100, 50, 20, 10, 5, 2 and 1 µg/L of chlorite, bromate, chlorate and bromide is prepared from 10 mg/L single standard solutions, once in deionized water and once in ADW. Additionally, two mixed anion standards for the calculation of the method detection limit MDL_S in ADW and deionized water are prepared from the 10 mg/L single standard solutions. Table 4 lists the concentrations of the mixed anion standards used to calculate the method detection limits (MDL_S), as well as the concentrations of the Quality Control Sample (QCS) in ADW, which are analyzed to determine retention time stability and peak area precision of the instrument.

Table 4. Concentrations of the MDL_S Calculation Standards and QCS Standard.

Analyte	MDL_S Calculation Standard (µg/L)	MDL_S Calculation Standard (µg/L) in Artificial Drinking Water	QCS for Precision (µg/L)
Chlorite	10	10	50
Bromate	15	20	50
Chlorate	20	20	500
Bromide	15	10	500

The standards to determine the linear calibration range are also diluted in ADW. Eight mixed calibration standards containing concentrations of 50, 20, 10, 5, 2, 1, 0.5 and 0.2 µg/L of Chlorite and Bromate and 500, 200, 100, 50, 20, 10, 5 and 2 µg/L of chlorate and bromide are prepared and injected. All concentration levels were prepared from the 10 mg/L standard solutions by diluting the respective volumes to 50 mL with artificial drinking water. To assess the performance of the chromatographic system, an External Control Sample (ECS) is prepared at the same concentration as the QCS. To prepare the ECS, 1000 mg/L stock solutions are prepared from chemicals other than the standard stock solutions used for the determination of the linear calibration range. The amounts to be used are given in Table 2. Like the QCS, the ECS is also diluted in ADW.

Eluent solutions

The eluent is prepared from stock solutions with concentrations of 1.00 mol/L. For the sodium carbonate stock solution, fill 53.00 g of Na_2CO_3 into a 500 mL volumetric flask and add 400 mL of deionized water. Shake the flask vigorously until the solution is completely clear. Let the solution cool to room temperature before filling the flask to the mark with deionized water. Mix the solution thoroughly once more. For the sodium bicarbonate stock solution, fill 42.00 g of $NaHCO_3$ into a 500 mL volumetric flask and follow the same procedure as for the Na_2CO_3 solution.

For the chromatographic system discussed here, the following eluent is prepared: 1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$ – To prepare this eluent, add 3.2 mL of the 1.0 mol/L Na_2CO_3 stock solution as well as 3.0 mL of the 1.0 mol/L $NaHCO_3$ stock solution into a 2000 mL volumetric flask, and fill to the mark with deionized water. Mix the solution thoroughly and filter the eluent over a 0.45 µm regenerated cellulose filter disc. Transfer the eluent to the S7150 Reagent Organizer and pressurize the container with inert gas (optional).

Preparation of the IC system

To achieve reproducible results, the system, especially the electrochemical suppressor unit, has to be equilibrated. When first installing the system, or if the system has not been in use for more than one week, the suppressor module has to be carefully prepared for analysis. To do so, apply a flow rate of 0.3 mL/min of water for 15 minutes to hydrate the eluent and regeneration channels of the suppressor. Let it rest for another 30 minutes. In the meantime, install the separation column (including the guard column) and switch on the column oven at 35 °C. Then switch to eluent and slowly increase the flow rate to 1.0 mL/min over the cause of 30 min. Switch on the current of the electrochemical suppressor. For a faster equilibration, a higher suppressor current of 150 mA can be applied for 2 hours at the desired flow rate of 1.0 mL/min. Ideally, the system is equilibrated overnight at the conditions used for analysis (1.0 mL/min, 25 mA). The baseline noise of the equilibrated system should be <2 nS/cm. After finishing the analyses, rinse the system (without column) with deionized water for 15 min, before shutting it down. If the system has been out of use for only a few days, the hydration step can be omitted. In this case, the equilibration can be started from the point after installing the column. If the system is in use on a daily basis, apply a lower flow rate of 0.1 mL/min and a current of 5 mA in downtimes, in-between analyses. This will ensure, that the system is equilibrated and ready-to-use within 1 hour after increasing the flow rate and suppressor current to the desired values. Make sure that the washing solution of the automatic sample injector is sufficiently filled with deionized water and perform two washing steps each at the injection port as well as at the washing port prior to analysis. During analysis, one washing step each at both ports is recommended after performing each injection. For more detailed instructions, please refer to the corresponding installation, maintenance and operator´s manuals of the instruments as well as the chromatography software. If using new columns, install the column and condition it at a flow rate of 0.3 mL/min before slowly increasing the flow rate to the desired value (1.0 mL/min).

Sample Preparation

After collecting the samples, or after opening of the bottled mineral water samples respectively, all samples have to be stabilized adding the appropriate amount of preservation solution (end concentration of ethylene diamine: 50 mg/L). All drinking water samples have to be filtered through an appropriate syringe filter (0.45 µm regenerated cellulose) discarding the first 1.0 mL of the filtrate. The samples have to be stored at 4 °C and analyzed within 24 hours after collection/ opening of the bottle.

Results and Discussion

The method validation for the determination of the disinfection byproducts chlorite, bromate and chlorate, as well as bromide, in drinking waters and bottled mineral waters was performed according to EPA method 300.1 (Revision 01, Part B).[11] All requirements of this method as well as ISO EN 15061:2001-12[12] and ASTM D6581-18[14] regarding quality control parameters are met. The methods state that optional columns and varying chromatographic conditions may be used to improve separation or lower the costs of measurements. Here, the Sykam A10 column with AGC-06 guard column and the Sykam S151-AG+ IC system were used for the validation, applying the chromatographic conditions listed above. The following quality control parameters were tested:

- Method detection limits MDL_S[15]
- Linearity (Linear Calibration Range, LCR)[16]
- Precision, by repeated injection of a Quality Control Sample (QCS)
- Verification of calibration standards and instrument performance, by injection of an External Control Sample (ECS)
- Laboratory performance, by injection of a laboratory reagent blank (LRB) and laboratory fortified blank (LFB)
- Analysis of laboratory duplicates
- Assessment of analyte recovery, by injection of Laboratory Fortified Matrices (LFM) for each sample

Instrument performance was checked throughout the entire sequence by analysis of initial, continuing and end calibration check standards.

Under consideration of the maximum contaminant levels for bromate of 10 µg/L, set by the corresponding regulatory authorities in the EU[8] and the US,[9] this method was developed and optimized to achieve a detection limit for bromate of less than 5 µg/L. A Sykam A10 column with AGC-06 guard column was used resulting in an good peak separation of bromate and the nearby eluting chloride peak. To enable the separation of disinfection byproducts and standard anions in the same run, the Sykam A10 is filled with a hydrophilic weak anion exchange resin based on an EVB-DVB copolymer with a particle size of 9 µm. ISO EN 15061:2001-12 requires the peak resolution (R) of all disinfection byproducts to be greater than 1.3 in artificial drinking water with a Cl^- and SO_4^{2-} concentration of 50 mg/L, if analyzed at their highest calibration levels of 50 µg/L for chlorite and bromate and 500 µg/L for chlorate and bromide respectively.[12] In addition to these requirements, a study was performed to examine the recovery of bromate and other analytes in the presence of high concentrations of chloride and sulfate, as they are often found in natural drinking waters. Peak resolution, retention time and peak symmetry of the quality control sample (50 µg/L ClO_2^-/BrO_3^-, 500 µg/L ClO_3^-/Br^-) in artificial drinking water are listed in Table 5. The retention times of all analytes are slightly shorter than for the method presented in AN12, while the resolution is comparable. The peak symmetries for the method presented in the application update are slightly better.

Table 5. Column performance parameters of the Sykam A10 at the chromatographic conditions listed above and concentration levels of the Quality Control Sample in Artificial Drinking Water.

Analyte	Retention time (min)	Resolution R	Peak Symmetry
Fluoride	4.47	–	1.05
Chlorite	6.20	6.33	1.03
Bromate	6.66	1.35	1.20
Chloride	7.88	2.63	0.62
Nitrite	9.44	3.27	1.09
Chlorate	10.98	3.48	1.03
Bromide	11.71	1.49	1.11
Nitrate	13.43	3.27	1.31
Phosphate	32.81	18.92	1.01
Sulfate	39.04	4.01	0.90

Figure 1 shows the separation of the inorganic anions found in the quality control sample of artificial drinking water, together with the disinfection byproducts chlorite, bromate, and chlorate. Figure 2 shows the separation of the disinfection byproducts at the concentration levels of the MDL_S-calculation standard in both deionized water and artificial drinking water.

Column: Sykam A10 (250 x 4.0 mm) + AGC-06 (50 x 4.6 mm)
Eluent: 1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 100 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (25 mA)

Peaks:		
	1. Fluoride	1.0 mg/L
	2. Chlorite	0.05
	3. Bromate	0.05
	4. Chloride	50.0
	5. Nitrite	0.1
	6. Chlorate	0.5
	7. Bromide	0.5
	8. Nitrate	30.0
	9. System Peak	
	10. Phosphate	0.1
	11. Sulfate	50.0

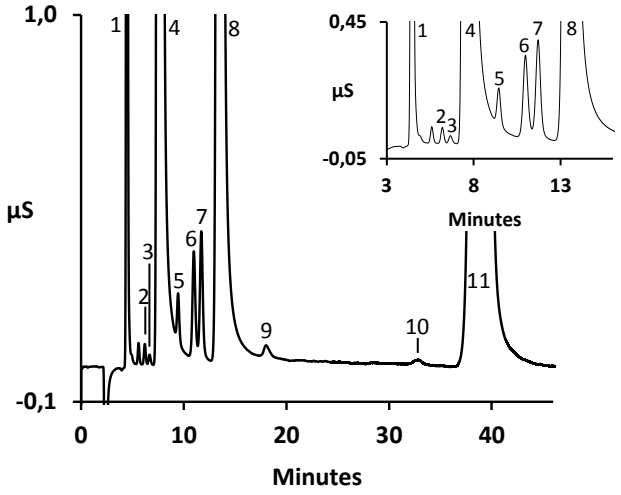

Figure 1. Separation of inorganic anions in the quality control standard in artificial drinking water on a Sykam A10.

Method Detection Limit MDL_S

As first part of the method validation, the method detection limit based on sample injection (MDL_S) was estimated for chlorite, bromate, chlorate and bromide. This was achieved by injecting seven mixed anion standards with the following concentrations: 100, 50, 20, 10, 5, 2 and 1 µg/L. The mixed anion standards were prepared twice, once in deionized water and once in artificial drinking water, to assess the influence other anions present in drinking water samples may have on the recovery of the disinfection byproducts and bromide. For each anion, the concentration at which the signal-to-noise ratio lies between 3 and 5 was determined or extrapolated.

The estimated method detection limits were multiplied by a factor of 5 to obtain the final concentrations of the MDL_S-calculation standard (Table 4). The MDL_S for each anion was determined by performing seven replicate injections of the MDL_S-calculation standard. The MDL_S is then calculated as $MDL_S = t \times SD$, where t is the Student´s value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.142613 for seven replicates) and SD is the standard deviation of the replicate analysis of the MDL_S-calculation standard for each anion. As expected, the method detection limits of chlorite and bromate in deionized water were significantly lower than in artificial drinking water, due to interference by the standard anions. The detection limits of chlorate and bromide however, are less affected by the presence of the other ions and were even found to be lower in artificial drinking water than in deionized water. For bromate, a method detection limit of 1.56 µg/L was achieved in deionized water and 1.80 µg/L in artificial drinking water. The MDL_S

for all other ions were also found to be within the range of up to 3 µg/L. Due to the lower injection volume of only 100 µL compared to 350 µL used for the method described in AN12, the detection limits of the DBPs and bromide were higher for the method presented here, as expected. Overall, this difference was found to be much more significant for the MDL_S in deionized water than in artificial drinking water. Surprisingly, for this method, the MDL_S in artificial drinking water was only higher for chlorite while it was lower for all other analytes.

Also, regarding the disinfection byproducts, all method detection limits are well below the maximum contaminant levels of chlorite, bromate and chlorate as defined in the EU drinking water directive[8] or the US EPA National Primary Drinking water regulations.[9] These are 10 µg/L for bromate (EU and US) and 0.25 mg/L for chlorite and chlorate (EU). The US EPA limits the content of chlorite in drinking waters to 1.0 mg/L, while chlorate is not part of the Primary Drinking Water Regulations.[17] The MDL_S values found in this study are listed in Table 5. In contrast to the MDL_S, the MDL_B is based on the injection of blanks. It applies, if the injection of the Laboratory Reagent Blank (LRB) gives a numerical result for one of the investigated anions. Here, the MDL_B was not determined, since none of the seven replicate injections of the laboratory reagent blank (LRB) showed any result for the investigated anions.[15]

Column: Sykam A10 (250 x 4.0 mm) + AGC-06 (50 x 4.6 mm)
Eluent: 1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 100 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (25 mA)

Peaks:			
	1. Fluoride		
	2. Chlorite	10.0	10.0 µg/L
	3. Bromate	15.0	20.0
	4. Chloride		
	5. Nitrite		
	6. Chlorate	20.0	20.0
	7. Bromide	15.0	10.0
	8. Nitrate		
	9. system Peak		
	10. Phosphate		
	11. Sulfate		

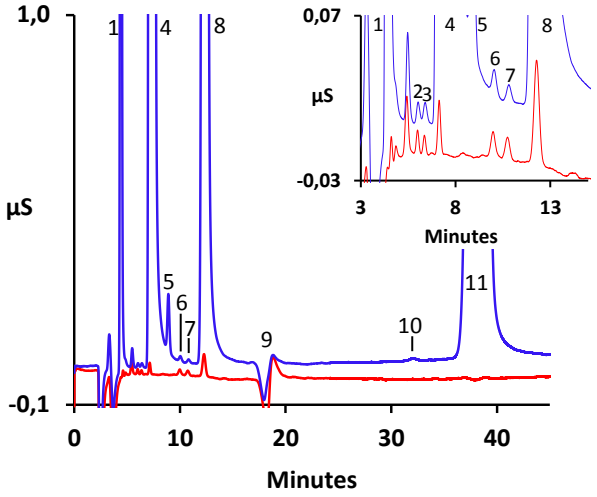

Figure 2. Separation of disinfection byproducts and bromide in the MDL_S-calculation standard diluted in deionized water (red) and artificial drinking water (blue)

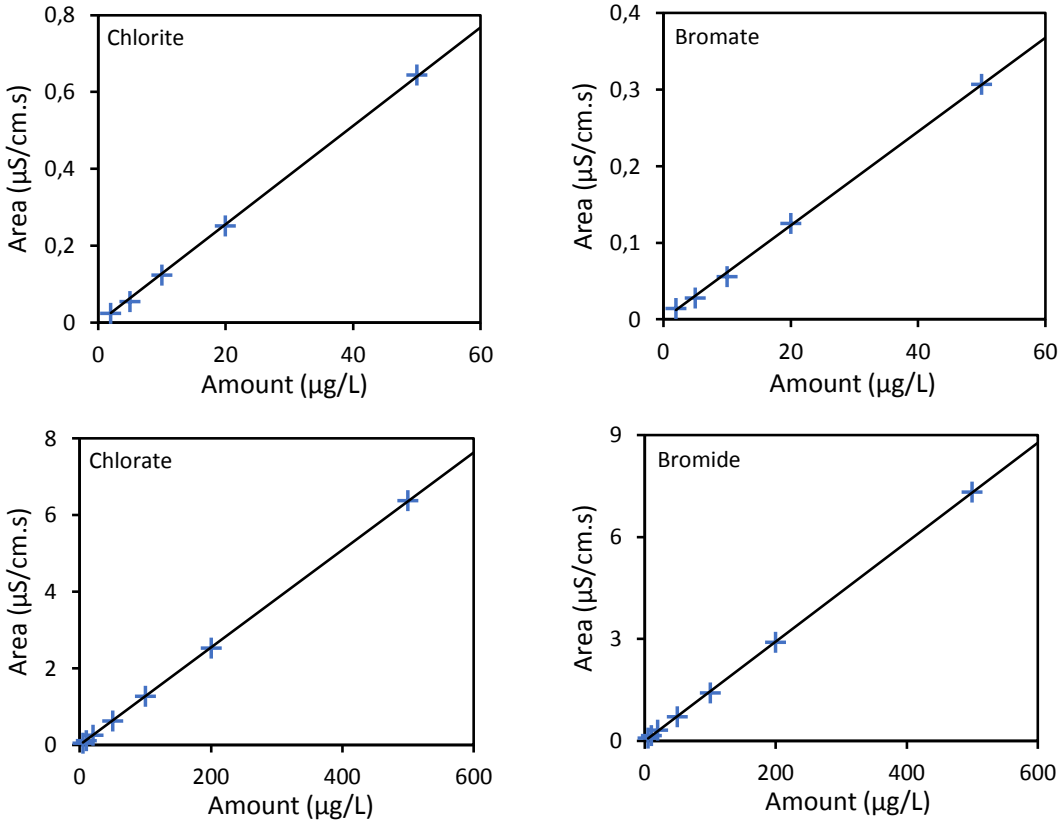

Figure 3. Calibration plots of the disinfection byproducts and bromide indicating the LCR used for calibration of the samples.

Table 5. Linearity, MDL_S in deionized water as well as in artificial drinking water (ADW), retention time- and peak area precision.

Analyte	Calibration range (µg/L)	Linearity (r^2)	Calculated MDL_S (µg/L) in H_2O	Calculated MDL_S (µg/L) in ADW	Retention Time Precision (RSD, %)	Peak Area Precision (RSD, %)
Chlorite	2–50	0.9999	1.07	1.54	0.08	0.96
Bromate	2–50	0.9997	1.56	1.80	0.08	1.46
Chlorate	5–500	1.0000	2.57	1.90	0.07	0.30
Bromide	5–500	1.0000	3.25	2.66	0.07	0.48

Linearity (LCR)

To assess the linear calibration range for each anion, mixed anion standards at eight calibration levels, covering the linear calibration range (LCR) for all disinfection byproducts and bromide were injected (Table 5). The seven calibration solutions were prepared at concentrations of 50, 20, 10, 5, 2, 1, 0.5 and 0.2 µg/L of chlorite and bromate and 500, 200, 100, 50, 20, 10, 5 and 2 µg/L of chlorate and bromide. The lowest calibration level was not evaluation, because none of the analytes could be integrated reliably. For the calibration of chlorite and bromate, only five calibration points, covering the range of 2 – 50 µg/L, were used for the calibration curve, since the chromatograms of the lower concentration levels (1 µg/L and 0.5 µg/L) exhibited a signal-to-noise ratio too low to be reliably integrated. Figures 3 and 4 show the corresponding chromatograms of the seven calibration solutions, as well as the linear calibration plots for the DBP oxyhalides and bromide, using the Sykam A10. Table 5 lists the linear calibration range as well as the correlation factors r^2 of the linear fit for all analytes. All linear fits exhibit correlation factors of $r^2 > 0.999$ and are considered very accurate. The calibration ranges used are the same as in AN12 and the correlation factors are comparable or even better than for the method described there.

Column: Sykam A10 (250 x 4.0 mm) + AGC-06 (50 x 4.6 mm)
Eluent: 1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 100 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (25 mA)
Peaks: 1. Fluoride 2. Chlorite 3. Bromate 4. Chloride 5. Nitrite 6. Chlorate 7. Bromide 8. Nitrate

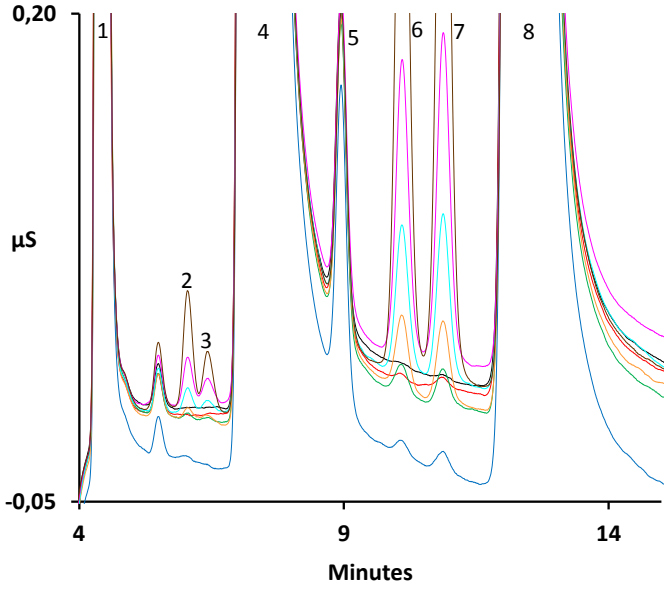

Figure 4. Chromatograms of the eight calibration solutions for linearity assessment.

Precision (QCS) and Instrument Performance (ECS)

Retention time and peak area precision was determined from seven replicate injections of a Quality Control Standard (QCS). Ideally, the concentrations of the standard anions in the QCS are selected to be very similar to those found in the field samples. Therefore, the QCS was prepared in artificial drinking water, the composition of which is listed in table 3. The concentrations of chlorite and bromate were 50 µg/L, whereas 500 µg/L of chlorate and bromide were added to the QCS to determine the precision of the instrument (Table 4). Table 5 shows the results of the Relative Standard Deviation (RSD) of retention time and peak area after seven replicate injections of the QCS. The RSD of the retention time is well below 1% and the RSD of the peak area was found to be no more than 2% for the investigated anions. All RSD values of this method were lower than for the method described in AN12.

If no new calibration is performed prior to analysis, the verification of the calibration standards and acceptable instrument performance is checked by the preparation and analysis of a QCS. Here, a new calibration was performed and the instrument performance was verified by the analysis of an External Calibration Standard (ECS). The ECS is prepared from 1000 mg/L single anion standard stock solutions, which are prepared from a different source than the standard stock solutions used for the calibration. The QCS, however is prepared from the same standard stock solutions that were used for the preparation of the calibration standards. If used for the verification of the instrument performance, the acceptance range for the QCS, and for the ECS in comparison to the QCS is ±15%. Table 6 shows the Relative Percent Difference (RPD) of the ECS, prepared from the external source stock solutions, and the QCS, prepared from the stock solutions of the calibration. The found differences are below 4.0% and thus well within the specified deviation range of ±15%. The RPD of chlorite is slightly higher than for the method described in AN12, but still quite low, while the RPD for all other analytes is comparable.

Table 6. Relative Percent Differences (RPD) of QCS and ECS.

Analyte	RPD (%)
Chlorite	3.1
Bromate	0.7
Chlorate	1.4
Bromide	1.7

Sample Analysis

The two drinking water samples, as well as the four bottled mineral water samples, were analyzed regarding their content of disinfection byproducts and bromide. As expected, no chlorite, bromate or chlorate were detected, since none of the water samples were treated with disinfecting agents like chlorine, chlorine dioxide, chloramine or ozone. Only bromide, which is the precursor ion for bromate formation during ozonation of drinking water, could be detected in all samples, however mostly in very low concentrations near the minimum reporting level (MRL). The MRL is defined as the lowest concentration at which an analyte can be quantified and is both higher or equal to the lowest calibration concentration, as well as higher than the MDL. Table 7 gives an overview of the MRLs for each anion based on the applied linear calibration range and the calculated MDLs. The analysis results for all samples are summarized in Table 8. Figures 5 and 6 show typical chromatograms of both fortified and unfortified samples, obtained from the analysis of drinking water from a municipal water supplier and a commercially available bottled mineral water.

Table 7. Minimum Reporting Levels based on the LCR and MDL.

Analyte	MRL (µg/L)
Chlorite	2.0
Bromate	2.0
Chlorate	5.0
Bromide	5.0

As required by US EPA method 300.1, all samples were analyzed as initial sample and laboratory duplicate. The values found in the laboratory duplicates were very similar to the respective initial samples, indicating the robustness of the validated methods. The Relative Percent Difference (RPD) of the laboratory duplicates, compared to the initial samples, is listed in Table 8. The RPDs could only be determined for bromide, as the disinfection byproducts were not present in the samples.

The required RPD for duplicate analyses according to EPA 300.1 is ±20% for concentrations up to 10x MRL, and ±10% for concentrations reaching from 10x MRL to the highest calibration level.[11] All duplicate analysis results are found to be well within the declared limits with an RPD of no more than 2.0%.

Table 8. Analysis results (µg/L) and RPD (%) of laboratory duplicate analyses.

Analyte	Drinking Water Milbertshofen	Drinking Water Penzberg	Mineral Water 1
Chlorite	n.d.	n.d.	n.d.
Bromate	n.d.	n.d.	n.d.
Chlorate	n.d.	n.d.	n.d.
Bromide	7.6 (1.3)	7.9 (-1.4)	14.3 (-1.2)
Analyte	**Mineral Water 2**	**Mineral Water 3**	**Mineral Water 4**
Chlorite	n.d.	n.d.	n.d.
Bromate	n.d.	n.d.	n.d.
Chlorate	n.d.	n.d.	n.d.
Bromide	39.3 (-0.8)	58.6 (1.5)	9.3 (2.0)

Initial, continuing, and end calibration check standards were injected before the first sample, after ten injections each, and at the end of the sequence, using the 100% calibration standard as check standard. For evaluation, the values of each anion of both the calibration standard and the check standard were directly compared to each other. The EPA 300.1 requirement for the deviation of the check standard is ±15%.[11] During the validation, the check standards did not differ by more than 7.9% from the corresponding calibration standard.

Analyte Recovery in Fortified Water Samples

Method performance for environmental analysis is typically validated by single- and multi-operator precision and bias studies on fortified samples (Laboratory Fortified Matrix, LFM). Tables 9 and 11 show the obtained recovery results for single-operator data for the disinfection byproducts and bromide spiked into drinking water and bottled mineral water samples. The samples were spiked with 10 mg/L solutions of the corresponding anions, which were prepared from the standard stock solutions used for the calibration. As the disinfection byproducts were not detected during sample analysis, concentrations of 5x MRL were added to all samples. In the case of bromide, concentration levels lower than 5x the found concentration were added according to the requirements set by US EPA method 300.1. Additionally, a Laboratory Blank (LFB) is fortified with disinfection byproducts and bromide at the highest concentrations used for the LFMs. Table 9 summarizes the recovery data found in the Laboratory Fortified Blank.

Table 9. Recovery data of the Laboratory Fortified Blank (LFB).

Analyte	LFB – Recovery	
	Amount added (µg/L)	Recovery (%)
Chlorite	10	114.8
Bromate	10	96.0
Chlorate	25	87.7
Bromide	60	103.0

The specification for the recoveries given in EPA Method 300.1 is ±25% for the LFM and ±15% for the LFB, if the fortification level of the LFB is <10x MRL.[11] The recovery data of the LFB as well as of the part of the drinking water samples is well within the specified range.

Effects of column overload on analyte recovery

Drinking water samples often contain high amounts of chloride and/or sulfate. This can cause column overload, which affects the separation and therefore the recovery of trace analytes such as ClO_2^-, BrO_3^-, ClO_3^- and Br^-. To assess the influence this may have on recovery values, a study with samples of enhanced salinity was performed. Fortified samples of Mineral Water 2 were prepared with 50, 100, 150, 200 and 250 mg/L of chloride or sulfate respectively, as well as 50, 100, 150 and 200 mg/L of chloride and sulfate. In these fortified samples, the recovery values of the spiked disinfection byproducts and bromide were examined. 25 µg/L of chlorite and bromate, and 50 µg/L of chlorate and bromide were added. Table 10 summarizes the recovery results for all samples.

Column: Sykam A10 (250 x 4.0 mm) + AGC-06 (50 x 4.6 mm)
Eluent: 1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 100 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (25 mA)

Peaks:			
	1. Fluoride	-	-
	2. Chlorite	n.d.	10.3 µg/L
	3. Bromate	n.d.	8.83
	4. Chloride	-	-
	5. Chlorate	n.d.	25.9
	6. Bromide	7.57	33.3
	7. Nitrate	-	-
	8. System Peak		
	9. Sulfate	-	-

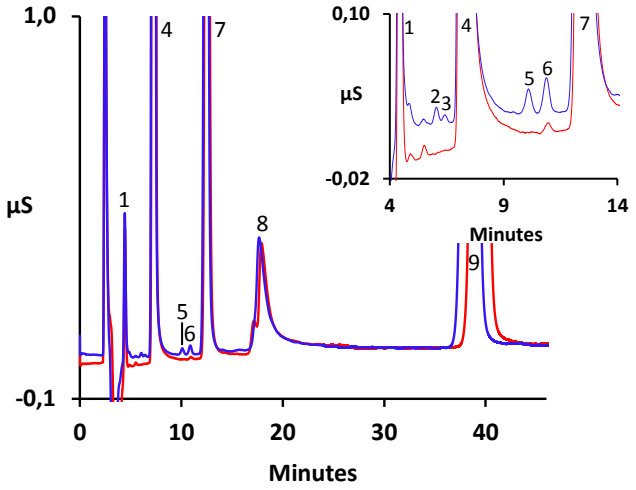

Figure 5. Determination of DBPs in drinking water from the municipal water supplier in Milbertshofen (Munich): unfortified sample (red) and fortified sample (blue).

Column: Sykam A10 (250 x 4.0 mm) + AGC-06 (50 x 4.6 mm)
Eluent: 1.6 mM Na_2CO_3, 1.5 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 100 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (25 mA)

Peaks:			
	1. Fluoride	-	-
	2. Chlorite	n.d.	8.93 µg/L
	3. Bromate	n.d.	9.76
	4. Chloride	-	-
	5. Chlorate	n.d.	25.2
	6. Bromide	9.33	33.0
	7. Nitrate	-	-
	8. System Peak		
	9. Sulfate	-	-

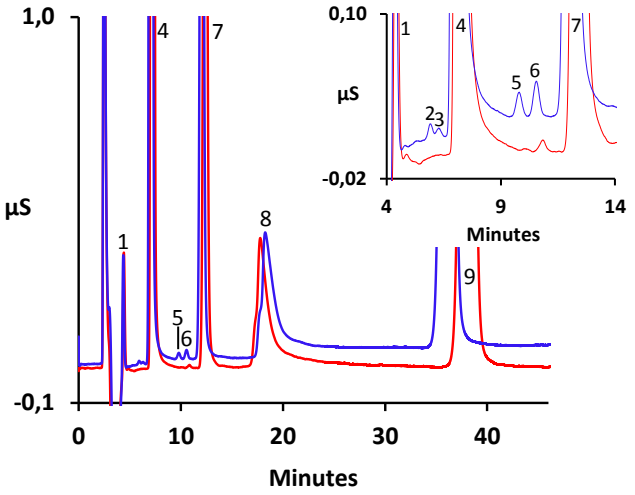

Figure 6. Determination of DBPs in bottled mineral water 4: unfortified sample (red) and fortified sample (blue).

Table 11. Recovery data from fortified drinking water and bottled mineral water samples using the Sykam A10 column.

Analyte	Drinking Water Milbertshofen		Drinking Water Penzberg		Mineral Water 1	
	Amount added (µg/L)	Recovery (%)	Amount added (µg/L)	Recovery (%)	Amount added (µg/L)	Recovery (%)
Chlorite	10	102.9	10	95.2	10	91.3
Bromate	10	88.3	10	91.5	10	85.3
Chlorate	25	103.6	25	91.9	25	114.7
Bromide	25	103.1	25	102.6	25	106.0
Analyte	**Mineral Water 2**		**Mineral Water 3**		**Mineral Water 4**	
	Amount added (µg/L)	Recovery (%)	Amount added (µg/L)	Recovery (%)	Amount added (µg/L)	Recovery (%)
Chlorite	10	102.3	10	107.9	10	89.3
Bromate	10	101.9	10	107.2	10	97.6
Chlorate	25	98.1	25	103.9	25	100.7
Bromide	40	100.6	60	114.9	25	94.7

Very good recoveries of 99% to 107% were obtained for bromide, which means that this analyte can be quantified reliably in the presence of high amounts of chloride and/or sulfate. For the other analytes, good recoveries of 93–105% were obtained for all samples spiked with only sulfate. This indicates that high amounts of sulfate alongside low concentrations of chloride do not significantly affect the quantification of the disinfection byproducts. Increasing amounts of chloride however, lead to a pronounced decrease in DBP recovery from samples. This effect is particularly strong for chlorite and especially bromate, which both elute before chloride, while chlorate is less affected. At a level of 50 ppm of added chloride, the recovery for bromate is still within an acceptable range of 78%, but higher values quickly decrease the recovery, to a point where bromate can no longer be resolved. Additionally, while sulfate by itself only has a

small influence on the recoveries of the DBPs, it quickly lowers the recoveries of chlorite and bromate in the presence of chloride. As such, 50 ppm of Sulfate alongside 50 ppm of chloride lower the recovery values of chlorite and bromate to 77.6 and 38.6% respectively, with the effect only becoming stronger with increasing chloride and sulfate concentrations. The recovery values of chlorate and bromide on the other hand are only slightly decreased in the presence of both sulfate and chloride.

In summary, chlorite and bromate can reliably be quantified in samples with chloride concentrations <50 mg/L, while chlorate and bromide can be quantified in samples with chloride concentrations <200 mg/L using the described method without an additional dilution step.

Table 10. Recovery data from samples with high chloride and/or sulfate concentrations

Cl^-	SO_4^{2-}	Recovery (%)			
(ppm)	(ppm)	ClO_2^-	BrO_3^-	ClO_3^-	Br^-
50	–	90.6	78.1	96.2	101.3
100	–	(82.0)*	(37.5)*	88.7	106.3
150	–	(60.8)*	–*	85.6	106.3
200	–	(43.1)*	–*	82.1	104.0
250	–	(25.0)*	–*	71.4	105.7
–	**50**	100.7	101.0	95.6	100.6
–	**100**	103.4	102.5	104.2	101.7
–	**150**	102.0	97.1	101.3	100.8
–	**200**	103.1	93.1	102.1	102.0
–	**250**	99.7	100.2	103.0	102.9
50	**50**	77.6	38.6	91.9	99.6
100	**100**	(60.2)*	–*	89.1	100.2
150	**150**	(43.1)*	–*	83.6	105.9
200	**200**	(29.6)*	–*	70.1	100.8

* Peaks are not resolved any more

The recoveries obtained for the method described in AN12 were quite good for chlorate and bromide, but in some cases the peaks were split into double peaks, which made them difficult to integrate and led to relatively low recoveries in some cases. This was not observed for the method described in this application update. The peaks for chlorate and bromide could be integrated easily and reliably and showed good peak shapes. On the other hand, the recoveries of chlorite and bromate were affected by significantly lower concentrations of chloride using this new method compared to the method described in AN12.

Summary

The Sykam A10 column provides suitable performance for the quantification of trace amounts of disinfection byproducts chlorite, bromate and chlorate as well as bromide, in drinking and bottled natural waters, as outlined in U.S. EPA Method 300.1,[11] ASTM D6581[14] and ISO EN 15061.[12] This was shown by a full method validation regarding all specifications given in the mentioned guidelines, concerning the determination of method detection limits, resolution, linearity and precision. Good recovery data for fortified sample matrices as well as the fortified blank were found using the Sykam A10 under the described chromatographic conditions. The column allows the analysis of trace bromate concentrations in samples with chloride concentrations up to 50 mg/L. The achieved method detection limit for bromate of 1.80 µg/L is well below the maximum contaminant level of 10 µg/L as stated by the EU and US drinking water authorities. Also, the maximum contaminant levels for chlorite and chlorate (0.25 mg/L in the EU and 1.0 mg/L in the US) can easily be reached during quantification, as the method detection limits for chlorite and chlorate were determined to 1.54 µg/L and 1.90 µg/L respectively. Therefore, the analytical method with the described chromatographic conditions is suitable for the analysis of disinfection byproducts in drinking waters of municipal water suppliers, as well as for quality controlling authorities in bottled mineral water industries.

The detection limits and all other quality control parameters of this method are comparable to those of the method described in AN12. Due to the use of only one A10 separation column with an AGC-06 guard column, it is possible to perform the determination of cations simultaneously, using the method for cation determination described in our application note AN09. Also, with the lower injection volume of 100 µL less sample volume is required than for the method described in AN12 that requires an injection volume of 350 µL, which reduces peak broadening. Due to the slightly shorter runtime and the possibility to perform simultaneous cation determination, this new method allows easier and faster analysis of drinking and mineral water samples. As only one A10 column is required this method is also more cost effective than the method described in AN12, which uses two A10 columns. Only for samples containing more than 50 mg/L of chloride it will be necessary to use the method described in AN12 to achieve reliable results for the determination of chlorite and bromate.

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