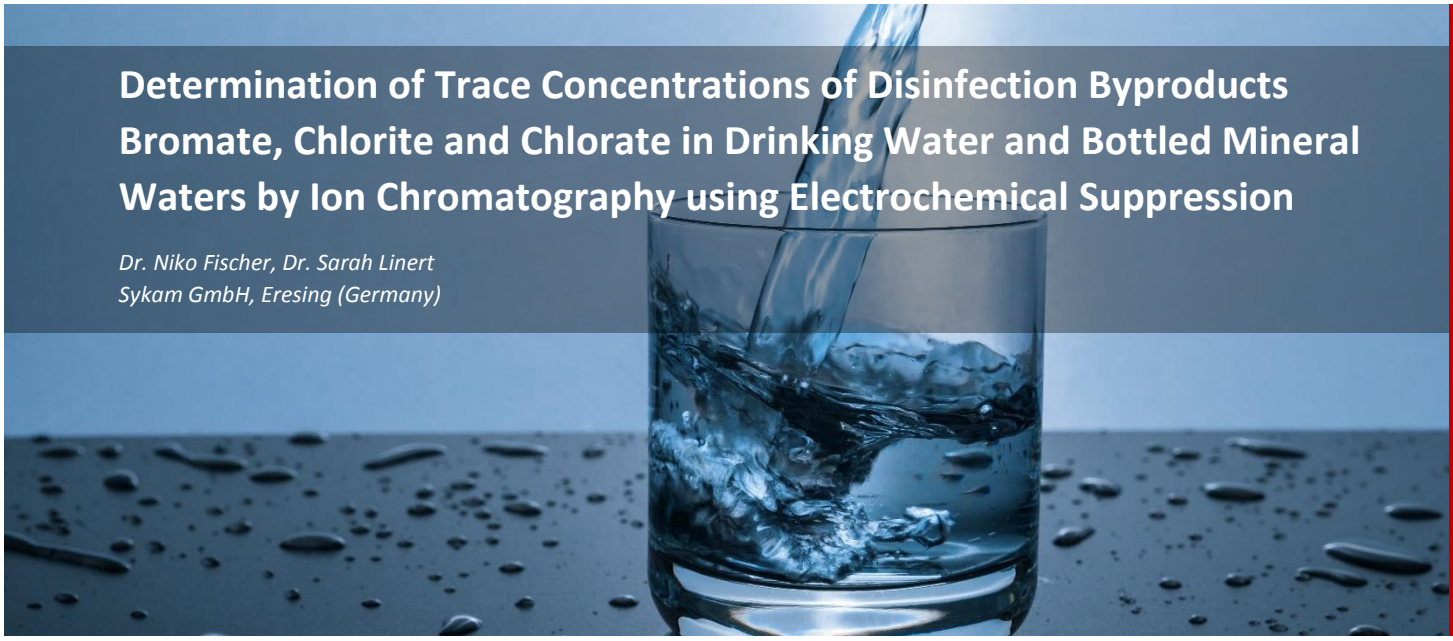

Determination of Trace Concentrations of Disinfection Byproducts Bromate, Chlorite and Chlorate in Drinking Water and Bottled Mineral Waters by Ion Chromatography using Electrochemical Suppression

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Introduction

A secured drinking water supply from public water systems in most countries is a self-evident responsibility of the public services. However, the availability of an uncritical water source for the public water supply regarding contamination with potentially dangerous microbes or other ingredients having adverse health effects after consumption, is not guaranteed everywhere. Hence, many water suppliers must treat their water using well established disinfection techniques such as the use of chlorine, chlorine dioxide, chloramine or ozone.[1,2] Moreover, the quality of bottled mineral water as a food product in the EU is regulated by the European Commission or the US Food and Drug Administration (FDA) in the United States respectively. The chemical disinfectants used for water treatment can react with naturally occurring inorganic or organic matter to form disinfection byproducts (DBPs) with undesired health effects after human consumption.[2] Chlorination, a technique, that is still widely used today can form trihalomethanes, haloacetic acids or chlorate,[3] whereas the treatment with chlorine dioxide is known to produce the oxyhalide DBPs chlorite and chlorate, which also applies for the use of chloramine as disinfectant.[4] To avoid taste and odor of treated drinking water, many suppliers use the treatment with ozone enriched air for disinfection. Ozone as a strong oxidant, however reacts with the naturally contained bromide to form bromate[5], which the International Agency for Research on Cancer has identified as a potential carcinogen.[6] The World Health Organization (WHO) has estimated an excess lifetime cancer risk of 10^{-4}, 10^{-5} and 10^{-6} for drinking water containing bromate at levels of 20, 2 and 0.2 µg/L.[7] Hence, many authorities like the European Commision (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, whereas Ion Chromatography (IC) has been established as the most common technique for their reliable quantification. The US EPA has published EPA Method 300.1,[11] which employs suppressed conductivity measurement for the analysis of DBPs in drinking water, whereas 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 this Application Note "Sykam AN12", the determination of the disinfection byproducts bromate, chlorite and chlorate as well as the naturally occurring precursor bromide in drinking water and bottled mineral waters by IC using suppressed conductivity measurement with electrochemical suppression is described. 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. Additionally, the Alternate Test Procedure (ATP) program of the EPA (Case No. D07-0012) approves the use of electrolytic eluent regeneration. For the described method two columns of the type Sykam A10 (250 x 4.0 mm) were coupled in series to achieve the desired resolution and the limit of detection of 2.64 µg/L for bromate, 1.09 µg/L for chlorite and 2.59 µg/L for chlorate, indicating, that the DBPs can easily be quantified down to the concentration levels of their respective MCLs, which were defined by the EC[8,10] and the EPA.[9] Next to the disinfection byproducts bromate, chlorite, chlorate and the precursor ion bromide, also the common inorganic anions fluoride, chloride, nitrite, nitrate, phosphate and sulfate can be resolved and determined in the same run using the described method, however, the quantification of these anions is omitted in this application note.

SYKAM CHROMATOGRAPHY

Equipment

The application note Sykam AN12 was designed for the use of a Sykam S151-AG+ IC module 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 module 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 the 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)
- Potassium bromate ($KBrO_3$, for analysis, EMSURE, ACS, ISO, Reag. Ph Eur), Merck (1.04912)
- Potassium chlorate ($KClO_3$, for analysis, EMSURE, ACS, Reag. Ph Eur), Merck (1.04944)
- 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 as well as four commercially available bottled mineral waters were analyzed (Table 1).

Table 1. List of samples analyzed.

No.	Name
1	Drinking Water (Augsburg)
2	Drinking Water (Steingaden)
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 expectedly none of the disinfection byproducts

is detected in any of the samples. However, resolution and recovery as well as any other system performance parameter was examined in this study. The two drinking water samples were collected in 250 mL PE-bottles with screw cap 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 analyzed as laboratory duplicates.

Chromatographic Conditions

Columns:	2x Sykam A10 (250 x 4.0 mm), Analytical Column and Guard Column
Eluent:	3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$
Flow Rate:	1.0 mL/min
Run Time:	52 min
Temperature:	35 °C
Injection Volume:	350 µL (full loop)
Detection:	Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor
Suppressor Current:	50 mA
Backpressure:	83 bar (1200 psi)
Base Conductivity:	15 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 for the seven standard anions as indicated above, if commercial standard stock solutions are not available. In case of chlorite, the standard solution needs to be titrated and its assay calculated before use, if prepared from the respective sodium salt, 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 not all of the four anions need to be analyzed, the respective 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)
Bromide	Sodium bromide (NaBr)	1.288
Bromate	Potassium bromate ($KBrO_3$)	1.306
Chlorate	Potassium chlorate ($KClO_3$)	1.469

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. All samples and standards are treated with EDA to prevent redox processes altering the found concentrations of the disinfection byproducts by adding 50 mg/L (e.g. 50 µL preservation solution/100 mL sample/standard) to the samples/standards.

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 diluted in Artificial Drinking Water (ADW) to simulate the interferences of the analytes chlorite, bromate, chlorate and bromide with other anions contained in the drinking water samples. Therefore, the composition of the artificial drinking water is close to that found in a typical drinking water sample. Table 3 summarizes the concentrations of the contained anions 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 10, 5, 2 and 1 µg/L of each chlorite, bromate, chlorate and bromide is prepared from 10 mg/L single standard solutions. 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 and are diluted in artificial drinking water and deionized water respectively. Table 4 gives the concentrations of the mixed anion standards prepared in ADW to calculate the method detection limits (MDL_S) as well as the concentrations of the Quality Control Sample (QCS) in ADW, which is analyzed to determine retention time stability and peak area precision of the instrument.

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

Analyte	MDL_S Calculation Standard (µg/L)	QCS for Precision (µg/L)
Chlorite	5	50
Bromate	10	50
Chlorate	25	500
Bromide	25	500

The standards to determine the linear calibration range are also diluted in ADW. Seven mixed calibration standards containing concentrations of 50, 20, 10, 5, 2, 1 and 0.5 µg/L of Chlorite and Bromate and 500, 200, 100, 50, 20, 10 and 5 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. For chlorite, no external chemical source was used but the commercially available standard stock solution was used instead. Similar to the QCS, the ECS is also diluted in ADW.

Eluent solutions

Eluents are prepared from eluent stock solutions with concentrations of 1.00 mol/L. For the sodium carbonate stock solution dissolve 53.00 g of Na_2CO_3 in 400 mL of deionized water in a 500 mL volumetric flask. Mix the solution thoroughly until completely clear and fill up the flask to the mark, when the solution has come to room temperature. Mix the solution thoroughly again. For the sodium bicarbonate stock solution dissolve 42.00 g of $NaHCO_3$ in 400 mL of deionized water in a 500 mL volumetric flask in the same manner by filling up the flask to the mark and shaking it vigorously.

For the chromatographic system discussed here, the following eluent is prepared: 3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$ – Add 6.4 mL of the 1.0 mol/L Na_2CO_3 stock solution as well as 6.0 mL of the 1.0 mol/L $NaHCO_3$ stock solution to 2000 mL of 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 and especially the electrochemical suppressor unit has to be equilibrated. When first installing the system or if the system has not been in use for longer time (several weeks), the suppressor module has to be carefully prepared before being ready for analysis. Therefore, apply a flow rate of 0.2 mL/min of water for 20 minutes, so that the eluent and regeneration channel of the suppressor are hydrated. Let it stand for another 20 minutes, meanwhile 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. 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, that are used for the analytical procedure regarding flow rate and suppressor current (1.0 mL/min, 50 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 10 min before shutting it down. If the system has not been in use for only a few days, the hydration step can be omitted starting with a low flow rate of eluent as described above. If the system is in use on a daily basis, apply a lower flowrate of 0.1 mL/min and a current of 5 mA after finishing the analyses, until the system is used again. 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 of 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 collection of 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 bottles.

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] so that 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 define, that optional columns and correspondingly different chromatographic conditions may be used to improve the separations or lower the costs of measurement. Here, two Sykam A10 columns together with the Sykam IC module S151-AG+ under the chromatographic conditions listed above were used for the validation, so that all required quality control parameters were tested including the determination of method detection limits MDL_S,[15] linearity (Linear Calibration Range, LCR),[16] precision by repeated injection of a Quality Control Sample (QCS), verification of the 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, and assessing 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. Therefore, two Sykam A10 columns were combined in series resulting in an excellent peak separation of bromate and the nearby eluting chloride peak, even at elevated chloride levels, whereas the first column serves as both, guard and analytical column. For the separation of disinfection byproducts and standard anions in the same run, the Sykam A10 is utilizing a hydrophilic weak anion exchange resin based on a EVB-DVB copolymer with a particle size of 9 µm. The peak resolution R of all disinfection byproducts if analyzed at their highest calibration levels of 50 µg/L for chlorite and bromate and 500 µg/L for chlorate and bromide respectively in artificial drinking water containing 50 mg/L Cl^- and 50 mg/L SO_4^{2-} is greater than 1.3 as required in the method performance section of ISO EN 15061:2001-12.[12] Additionally, a study was performed to examine the recovery of bromate and other analytes in the presence of high concentration levels of chloride and sulfate, as 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.

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	7.10	-	1.06
Chlorite	9.72	5.72	1.13
Bromate	10.42	1.34	1.29
Chloride	12.32	2.86	0.20*
Nitrite	14.70	3.52	1.45
Chlorate	16.69	3.09	1.54
Bromide	18.08	1.96	1.39
Nitrate	20.27	2.67	3.10
Phosphate	35.33	12.33	0.90
Sulfate	43.95	5.02	0.47*

*Chloride and Sulfate cause column overload at 50 mg/L and an injection volume of 350 µL.

Column: 2x Sykam A10 (250 x 4.0 mm)
Eluent: 3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 350 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 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. Phosphate	0.1
10. Sulfate	50.0

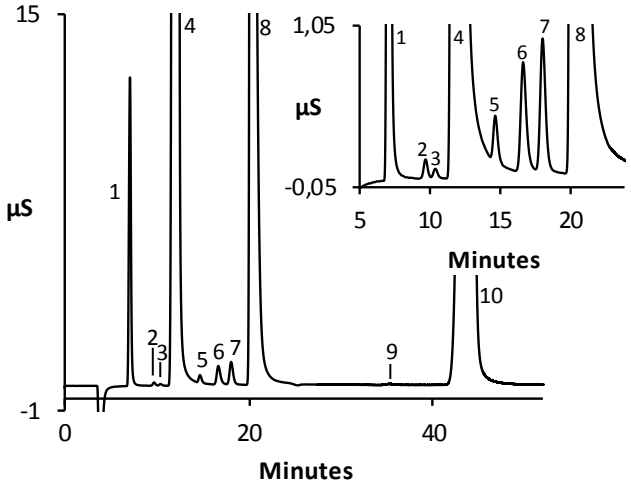

Figure 1. Separation of inorganic anions in the quality control standard in artificial drinking water on a Sykam A10.

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

Method Detection Limit MDL_S

First, the method detection limit based on sample injection (MDL_S) was estimated for chlorite, bromate, chlorate and bromide by the injection of four mixed anion standards with the following concentrations: 10, 5, 2 and 1 µg/L. The mixed anion standards were prepared in duplicate, one of them was diluted in deionized water, the second in artificial drinking water to assess the influence on the recovery of the disinfection byproducts and bromide, which other anions present in drinking water samples may have. For each anion, the concentration, at which the signal-to-noise ratio lies between 3 and 5 was determined or extrapolated.

Column: 2x Sykam A10 (250 x 4.0 mm)
Eluent: 3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 350 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)
Peaks:

1. Fluoride	
2. Chlorite	5.0 µg/L
3. Bromate	10.0
4. Chloride	
5. Nitrite	
6. Chlorate	25.0
7. Bromide	25.0
8. Nitrate	
9. Phosphate	
10. Sulfate	

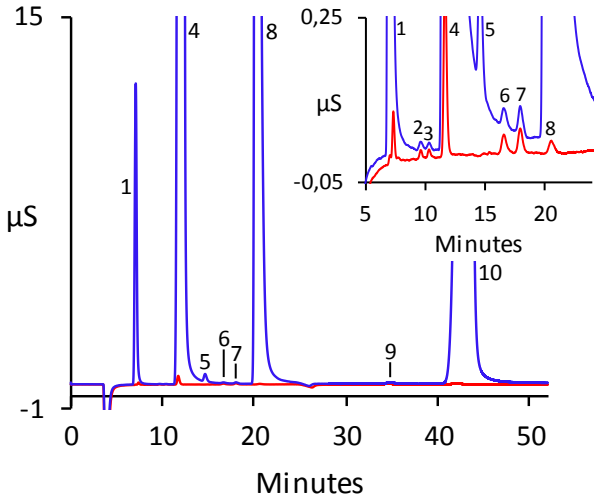

Figure 2. Separation of disinfection byproducts and bromide in the MDL_S-calculation standard diluted in deionized water (red) and artificial drinking water (blue).

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. Expectedly, the method detection limits in deionized water were significantly lower as if prepared in artificial drinking water. For bromate, a method detection limit as low as 1.13 µg/L could be achieved, whereas in artificial drinking water, the method detection limit could be determined to 2.64 µg/L. The MDL_S for all other ions are also in the range of up to 3 µg/L. Regarding the disinfection byproducts, all method detection limits are well below the maximum contaminant levels of chlorite, bromate and chlorate as directed in the EU drinking water directive[8] or the US EPA National Primary Drinking water regulations,[9] which are 10 µg/L for bromate (EU and US) and 0.25 mg/L for chlorite and chlorate (EU). The US EPA limited the content of chlorite in drinking waters to 1.0 mg/L, whereas 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]

Linearity (LCR)

To assess the linear calibration range for each anion, mixed anion standards at seven calibration levels covering the linear calibration range (LCR) for all disinfection byproducts and bromide as indicated in table 5, were injected. The seven calibration solutions were prepared at concentrations of 50, 20, 10, 5, 2, 1 and 0.5 µg/L of chlorite and bromate as well as 500, 200, 100, 50, 20, 10 and 5 µg/L of chlorate and bromide. For the calibration of chlorite and bromate, only five calibration points covering the range from 2 – 50 µg/L were used for the calibration curve, since the chromatograms of the two lowest 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.

Column: 2x Sykam A10 (250 x 4.0 mm)
Eluent: 3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 350 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)
Peaks: 1. Fluoride 5. Nitrite
2. Chlorite 6. Chlorate
3. Bromate 7. Bromide
4. Chloride 8. Nitrate

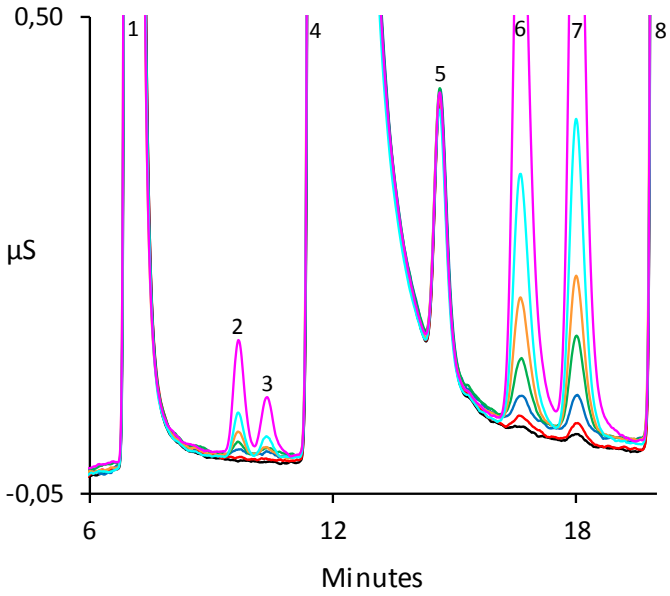

Figure 3. Chromatograms of the seven calibration solutions for linearity assessment.

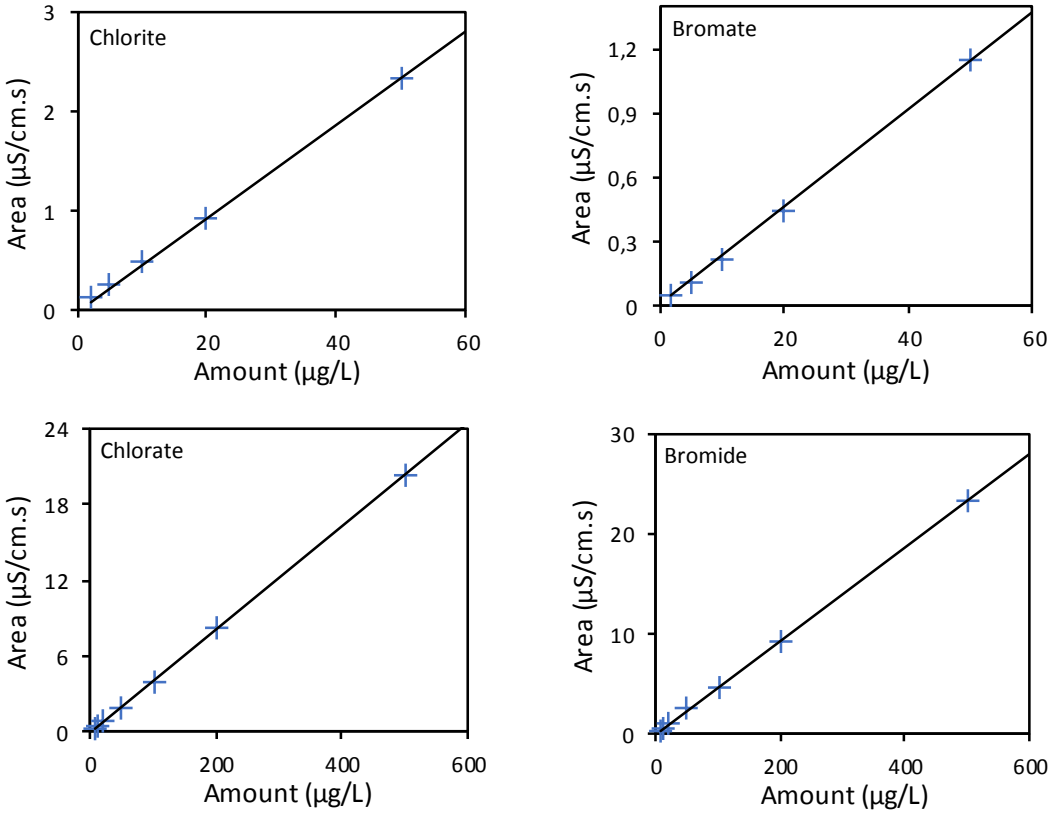

Figure 4. Calibration plots of the disinfection byproducts and bromide indicating the LCR used for calibration of the samples.

Table 5. Linearity, MDL_S in H_2O 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	0.9998	0.29	1.09	0.19	1.51
Bromate	2 – 50	0.9997	1.13	2.64	0.21	2.06
Chlorate	5 – 500	0.9999	1.99	2.59	0.22	0.44
Bromide	5 – 500	0.9998	1.48	2.98	0.21	0.72

Precision (QCS) and Instrument Performance (ECS)

Retention time and peak area precision was determined from seven replicate injections of a Quality Control Standard (QCS). The concentration of the standard anions in the QCS ideally is very similar to those found in the field samples. Therefore, the QCS was diluted in artificial drinking water, the composition of which is displayed in table 3. The concentration 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 not more than 2% for the investigated anions. If no new calibration is performed prior to analysis, the verification of the calibration standards and acceptable instrument performance is shown 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. (Exception: for chlorite, the commercially available 1000 mg/L stock solution was used). The QCS, however is prepared from the same standard stock solutions that were used for the preparation of the calibration standards. The acceptance range for the QCS, if used for the verification of the instrument performance 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 2.0% and thus well within the specified deviation range of ±15%.

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

Analyte	RPD (%)
Chlorite	-1.8
Bromate	+0.9
Chlorate	-1.8
Bromide	-1.9

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, in none of the examined water samples, chlorite, bromate or chlorate could be detected since none of the water samples was 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 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, which is higher or equal to the lowest calibration concentration and 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 obtained for the analysis of drinking water from a municipal water supplier and a commercially available bottled mineral water both as unfortified and fortified samples.

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

Column: 2x Sykam A10 (250 x 4.0 mm)
Eluent: 3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 350 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)

Peaks:			
	1. Fluoride	-	-
	2. Chlorite	n.d.	10.0 µg/L
	3. Bromate	n.d.	10.0
	4. Chloride	-	-
	5. Nitrite	-	-
	6. Chlorate	n.d.	26.4
	7. Bromide	12.2	52.9
	8. Nitrate	-	-
	9. Phosphate	-	-
	10. Sulfate	-	-

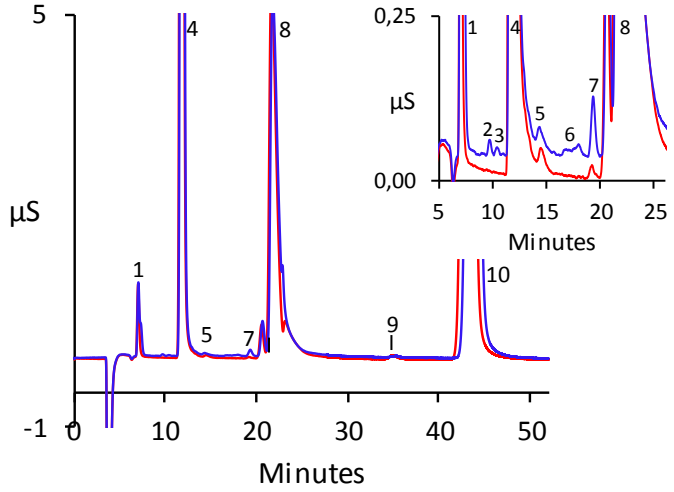

Figure 5. Determination of DBPs in drinking water from the municipal water supplier in Augsburg: unfortified sample (red) and fortified sample (blue).

All samples were analyzed as laboratory duplicates as directed in the US EPA method 300.1, however the values found in the laboratory

duplicates do not differ significantly from the respective initial samples indicating the robustness of the validated methods. The Relative Percent Difference (RPD) of the laboratory duplicates as compared to the initial samples is displayed in Table 8.

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.

Column: 2x Sykam A10 (250 x 4.0 mm)
Eluent: 3.2 mM Na_2CO_3, 3.0 mM $NaHCO_3$
Flow Rate: 1.0 mL/min
Inj. Vol.: 350 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)

Peaks:			
	1. Fluoride	-	-
	2. Chlorite	n.d.	8.36 µg/L
	3. Bromate	n.d.	10.5
	4. Chloride	-	-
	5. Nitrite	-	-
	6. Chlorate	n.d.	21.5
	7. Bromide	16.8	63.6
	8. Nitrate	-	-
	9. Phosphate	-	-
	10. Sulfate	-	-

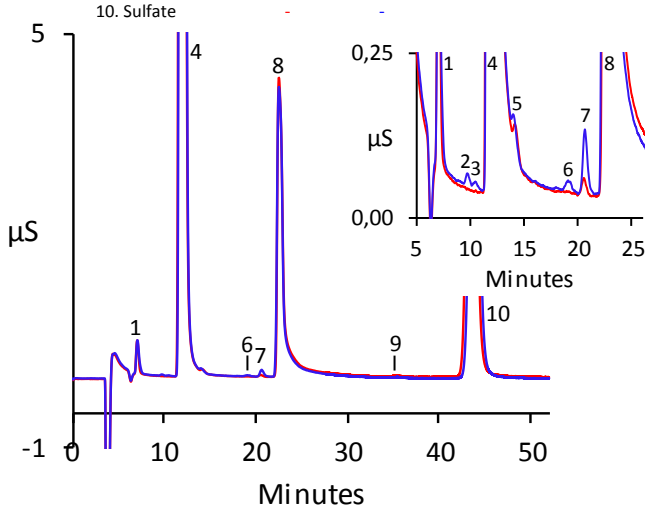

Figure 6. Determination of DBPs in bottled mineral water 4: unfortified sample (red) and fortified sample (blue).

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

Analyte	Drinking Water Augsburg	Drinking Water Steingaden	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	12.2 (2.3%)	24.6 (7.6%)	38.5 (6.5%)
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	28.7 (4.2%)	5.3 (-1.8%)	16.8 (0.3%)

Initial, continuing and end calibration check standards were injected at the beginning of the sample injections, after ten injections each during the sequence and at the end of the sequence, whereas the 100% calibration standard was used as check standard. 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 2.9% from the corresponding calibration standard. For evaluation, the areas under the curve of each anion of both, the calibration standard and the check standard, were directly compared to each other.

Analyte Recovery in Fortified Water Samples

The performance of methods used for environmental analysis are typically validated through 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 using two Sykam A10 columns 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 at concentrations of 5x MRL for all samples, in which the specified anion was not detected and at concentration levels lower than 5x the found concentration in case of bromide according to the requirements set by US EPA method 300.1. Additionally, a Laboratory Blank (LFB) is fortified at the same concentrations of disinfection byproducts and bromide. 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	99.9
Bromate	10	97.1
Chlorate	25	101.5
Bromide	50	102.5

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 largest part of the drinking water samples is well within the specified range. However, the recovery of chlorate in most samples is around 80%. One reason could be the influence of higher sulfate concentrations affecting the peak form of chlorate. The chlorate peak is progressively divided into a double-peak at higher sulfate concentrations making it difficult to reliably integrate the peak. Also high chloride concentrations affect the integration of the chlorate peak, since it elutes in the tailing of the chloride peak. More findings regarding the recovery of the disinfection byproducts depending on the chloride and sulfate content of the sample are discussed in the next chapter.

Effects of column overload on analyte recovery

Due to the large injection volume of 350 µL, which is necessary for the trace analysis of the discussed analytes, high concentrations of any of the standard anions, most likely chloride and sulfate, increasingly lead to column overload, which affects the separation and hence, the recovery of the trace analytes ClO_2^-, BrO_3^-, ClO_3^- and Br^-. To asses the influence, a study with samples of enhanced salinity was performed. Therefore, Mineral Water 3 was fortified with 50, 100, 150, 200 and 250 mg/L of chloride or sulfate respectively. Additionally, samples were fortified with 50, 100, 150 and 200 mg/L of chloride and sulfate. In these fortified samples, the recovery of the

spiked disinfection byproducts and bromide was examined. Chlorite and bromate were added at concentrations of 25 µg/L, whereas 50 µg/L chlorate and bromide were added. Table 10 summarizes the recovery results for all samples. At first sight, the values are randomly spread over a range between 66 and 115%, whereas most of the values are still within the specified range of ±25%. Chlorate recoveries tend to result in lower recoveries than observed for the other anions. Most importantly, it can be stated, that the peak form and hence, the integrability of the peaks are strongly influenced by the added amount of chloride or sulfate respectively. The peak pair chlorite/bromate is well resolved up to a chloride content of 100 mg/L, whereas at concentrations >100 mg/L the peaks are not separated any more making it impossible to quantify them. The sulfate content has no influence on the peak form and resolution of chlorite/bromate. The sulfate concentration, on the other hand is affecting the peak form of bromide. At sulfate concentrations of 150 mg/L or higher, the bromide peak is splitting into a double peak. The same can be observed for the chlorate peak at either high chloride or high sulfate concentrations. In summary, the disinfection byproducts and bromide can reliably be quantified in samples with chloride concentrations <100 mg/L and sulfate concentrations <150 mg/L, indicating, that the great majority of drinking water samples can be analyzed for disinfection byproducts and bromide 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		86.6	93.6	78.9**	96.6
100		91.7	106.8	71.4**	101.1
150		(89.5)*	(103.9)*	66.1**	93.8
200		-*	-*	72.1**	98.9
250		-*	-*	77.9**	90.0
	50	90.4	101.9	114.9**	86.0
	100	95.9	110.3	115.5**	85.7
	150	86.8	103.2	81.6	76.6**
	200	93.3	105.3	90.8	95.2**
	250	91.3	105.3	93.7	90.6**
50	**50**	85.6	84.6	91.2	95.3
100	**100**	96.9	115.5	82.1	92.3
150	**150**	(91.1)*	(105.2)*	99.7	78.4**
200	**200**	-*	-*	114.2	77.4**

*peaks are not resolved any more
**double peak formation

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

Analyte	Drinking Water Augsburg		Drinking Water Steingaden		Mineral Water 1	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Chlorite	10	100.5	10	97.8	10	99.2
Bromate	10	103.1	10	97.2	10	118.5
Chlorate	25	105.8	25	85.9	25	72.7
Bromide	50	81.7	50	97.1	50	84.1
Analyte	**Mineral Water 2**		**Mineral Water 3**		**Mineral Water 4**	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Chlorite	10	97.9	10	91.0	10	83.6
Bromate	10	92.7	10	101.9	10	105.2
Chlorate	25	82.1	25	81.7	25	86.1
Bromide	50	113.2	25	91.9	50	93.9

Summary

The Sykam A10 column provides suitable performance for the quantification of trace amounts of the disinfection byproducts chlorite, bromate, chlorate and 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 the fortified sample matrices as well as for the fortified blank were found using the Sykam A10 under the described chromatographic conditions. The column exhibits a high capacity making it possible to analyze trace bromate concentrations in samples of high ionic strength with chloride concentrations up to 100 mg/L and sulfate concentrations up to 150 mg/L. The achieved method detection limit for bromate of 2.64 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 1.09 µg/L and 2.59 µg/L respectively. The analytical method under the described chromatographic conditions hence 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.

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SYKAM CHROMATOGRAPHY